Geographic variation in behaviour and dim light adaptation in *Cyrba algerina* (Araneae, Salticidae)

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Abstract

Cyrba algerina is a salticid (Salticidae) spider that lives on the undersides of stones. Two populations were studied, Sintra and Algarve (Portugal), and shown to have similar phenology but different dominant prey. Life cycle in the laboratory was similar for the two populations, but Sintra matured at larger size than Algarve individuals, with these differences potentially having a genetic basis. Sintra individuals used prey-specific prey-capture behaviour against allopatric (*Oecobius amboseli*) and sympatric (*O. machadoi*, *Trachyzelotes bardiae*) spider and insect (bristletails) species. In contrast, Algarve *C. algerina* only adopted specialised capture behaviour against bristletails. Sintra, but not Algarve, individuals responded to the odour of *O. machadoi* and *T. bardiae*, and showed preference for *T. bardiae* over *O. machadoi.* Interpopulation variation in the use of specific prey-capture behaviour and in sensitivity to odour cues from prey is directly related to the prey available to individuals from each population, suggesting local adaptation to local prey. Preference for oecobiids seems to be controlled by an experience-triggered developmental switch. The optics and histology of *C. algerina*'s principal eye suggest that living in a microhabitat with dim ambient light has favoured sensitivity at the expense of spatial acuity. Short focal length, reduced power of the eye's diverging lens, and wide, contiguous rhabdomeres, seem to minimise the visual constraints imposed by the low light levels in *C. algerina*'s microhabitat. While relying solely on vision, *C. algerina* can detect, identify and capture prey in dim-light conditions under which other salticids perform poorly. *C. algerina*'s behaviour suggest use of temporal summation to improve its visual performance in dim light.

CHAPTER 1

Introduction

> "Our ignorance of the laws of variation is profound. Not in one case out of a hundred can we pretend to assign any reason why this or that part has varied. But whenever we have the means of instituting a comparison, the same laws appear to have acted in producing the lesser differences between varieties of the same species, and the greater differences between species of the same genus. Changed conditions generally induce mere fluctuating variability, but sometimes they cause direct and definite effects; and these may become strongly marked in the course of time, though we have not sufficient evidence on this head."
>
> Darwin 1859

In the past, behaviour was assumed to be largely invariant within species. Variation in behaviour was regarded as undesirable and confounding noise of little intrinsic value (see Magurran 1999, Verrell 1999). Gradually, as more studies documented variation in behaviour, this view seemed to change, and it is now widely accepted that geographic variation in behaviour may actually be the norm rather than the exception (Foster 1999, Foster & Endler 1999).

One of the major goals of behavioural ecology is to understand the capacity of natural populations to adapt to their local environment. A further goal is to investigate the specific selection pressures that drive the evolution of particular behaviour patterns. The comparative study of carefully selected populations is of particular interest, as it may provide useful insights into the specific causes of adaptive (and nonadaptive) differentiation in behaviour (Riechert 1999). Relatively to species, populations are likely to have separated more recently, and tend to differ in fewer traits than species. As a result, fewer confounding variables are expected when interpreting data from population comparisons than would be the case if comparing different species (Arnold 1992, Foster 1999, Foster & Endler 1999, Verrell 1999). Population comparisons are also important for their potential to provide insights on how the interaction between genes and environment might generate geographic variation in behaviour (Carroll & Corneli 1999, Foster & Endler 1999, Riechert 1999).

My research has been on a particular spider species, *Cyrba algerina* (Salticidae) a primitive jumping spider from the subfamily Spartaeinae (Wanless 1984, Maddison & Hedin 2003, Su *et al* 2007). Salticids have eight eyes, but it is the pair of large anterior-medial eyes (the 'principal eyes') that set salticids apart from all other spiders. The principal eyes have a unique combination of features, including telephoto optics, moveable eye tubes behind a fixed corneal lens, light guides in receptor cells and a very fine-grain sampling mosaic in the foveal region of the retina, providing salticid with spatial acuities exceeding that known for any other animal of comparable size (Land 1969a,b 1981, 1985, Land & Nilsson 2002). Spartaeines are of interest in salticid research for several reasons. Besides being a basal branch in the Salticidae family (Maddison & Hedin 2003, Su *et al* 2007), many spartaeines species, including *C. algerina*, are known to be versatile predators, using unusual and intricate vision-mediated behaviour by which they prey on other spiders (Forster 1982, Jackson & Hallas 1986a, Jackson & Pollard 1996, Harland & Jackson 2004).

A thoroughly studied example of interpopulation variation in behaviour comes from the spartaeine genus *Portia* (Jackson & Hallas 1986a, Jackson 1992, Jackson & Carter 2001, Jackson *et al* 2002a). Geographically separated populations of single species of *Portia* are known to adopt distinctively different predatory strategies that appear to be adaptively fine-tuned to local prey. Initial behavioural studies published on *C. algerina* from Portugal (the Algarve), Spain, Israel and Azerbaijan (Jackson & Hallas 1986b, Jackson 1990, 2002, Jackson & Li 1998, Cerveira *et al* 2003, Guseinov *et al* 2004) also seem to suggest the existence of substantial geographic variation in the predatory behaviour of this jumping spider species.

My goal in this thesis was to investigate how an unusual microhabitat, together with extensive variation in the prey types available over a wide geographic range, may have shaped the evolution of interpopulation variation in *C. algerina*'s predatory strategies.

Most Spartaeines have a primarily tropical distribution, but *C. algerina* occurs at higher latitudes, and its geographic distribution, stretching from the Canary Islands through the Mediterranean Region and into Central Asia, is the widest known for any spartaeine (Wanless 1984). In Chapter 2, I provide information on the phenology, habitat and the prey records of two populations of *C. algerina* in Portugal, the Algarve and Sintra. This information is important background for later chapters.

In Chapter 3, I describe the life cycle of Sintra and Algarve *C. algerina* in the laboratory and investigate whether observed body-size variation between the Algarve and Sintra individuals is based on genetic differences between the populations or whether, alternatively, this variation is entirely a consequence of environmental differences.

Recent work done on the Baku population of *C. algerina* in Azerbaijan showed that this population adopts a specific behaviour to capture a particular species, *Oecobius maculatus* (Oecobiidae), a common spider in this populations' habitat (Guseinov *et al* 2004). In Chapter 4, this is the rationale for investigating the prey-capture behaviour used by the Algarve and Sintra *C. algerina* against sympatric and allopatric spider and insect prey species. Particular attention is given to sympatric and allopatric oecobiid species.

The evolution of good eyesight and elaborate vision-based behaviour has not precluded proficiency by salticids at using other sensory modalities. In particular, numerous studies have demonstrated that chemical cues play important roles during both intra- and interspecific interactions (Pollard *et al* 1987, Taylor & Jackson 1999, Clark *et al* 1999, 2000, Jackson *et al* 2002b, Jackson *et al* 2005). Chapter 5 considers *C. algerina*'s sensitivity to the odour of sympatric and allopatric spider prey. *C. algerina* individuals from both populations were tested in a Y-shaped olfactometer to assess their response to volatile olfactory cues from sympatric and allopatric spider species (*O. machadoi* and *O. amboseli*, *Trachyzelotes bardiae* (Gnaphosidae)) and one sympatric insect species (a bristletail, *Ctenolepisma* sp. (Thysanura)).

In Chapter 6, I extend the work in Chapter 5 by investigating *C. algerina*'s ability, on the basis of odour cues alone, to choose between *O. machadoi* and *T. bardiae*.

In Chapter 7, I investigate whether the different sensitivity to the odour of *O. machadoi* shown by the Algarve and Sintra individuals in Chapter 5 is influenced by previous experience with prey or whether, on the contrary, it is strictly innate (i.e., whether no prior experience of the odour is required before the response is expressed). In this chapter the prey preferences of Sintra and Algarve populations of *C. algerina* were tested with sympatric and allopatric spider species in vision- and odour-based choice tests after a 7-day feeding period on one of three spider species (*O. machadoi*, *O. amboseli* and *Nephylengys* sp. I also considered the influence of oecobiid odour on *C. algerina*'s prey preferences by exposing *C. algerina* individuals exclusively to the odour of prey (i.e., in the absence of experience preying on oecobiids). The findings from this study are of interest in the context of associative learning, food imprinting and developmental switches.

Chapter 8 is concerned with *C. algerina*'s eyes. Remarkable variation in the details of retinal organization of salticid anterior median eyes has been documented within the Spartaeinae, in a series of studies from David Blest's laboratory (Blest & Sigmund 1984, 1985, Blest *et al* 1990). From an evolutionary perspective, the retinal ultrastructure of *C. algerina*'s anterior median eyes is considered to be less organised than that of typical ("advanced") salticid eyes and, consequently, it has been suggested that *C. algerina*'s anterior median eyes may represent

an intermediate stage in the evolution of the jumping spider eyes (Blest *et al* 1990), an hypothesis which seems consistent with a recent DNA-based phylogenetic study of the Spartaeinae (Su *et al* 2007).

C. algerina lives in a very particular microhabitat, the undersides of stones, a microhabitat which, when compared to that of most typical salticids, has very low ambient light levels. For a spider-size animal, the trade-offs between resolution and sensitivity are expected to be especially severe (i.e., deriving a more sensitive eye requires a loss in resolution: Land 1981, Land & Nilsson 2002). It is therefore expected that in the eyes of small species living in dimly lit habitats, sensitivity will be maintained at a cost of both the magnification-properties of the lens system and the spatial acuity supported by the sampling mosaic of the retina. In Chapter 8, I investigate the optics and the ultrastructure of *C. algerina*'s anterior median eyes and propose that dim ambient light levels, as well as a low diversity of prey found in *C. algerina*'s habitat, might have favoured the retention of a retinal mosaic that emphasizes sensitivity at the expense of spatial acuity. By having a short focal length, reduced power of the diverging component, wider and contiguous adjacent rhabdomeres, *C. algerina*'s principal eyes may be able to minimise the constraints imposed by the low light levels of its microhabitat.

In Chapter 9, I investigate how *C. algerina*'s predatory behaviour is affected by low ambient light levels. Orientation and mirror display tests were staged under dim light to determine the effect of decreasing light levels in *C. algerina*'s behaviour. Predatory encounters with other spider prey revealed that *C. algerina* can capture prey under low ambient light levels and additionally suggest that *C. algerina*'s eyes, compared to those of a representative typical salticid species (*Evarcha culicivora*), are more sensitive to light.

Finally, on Chapter 10, I provide a synthesis of the findings presented in the previous chapters.

My findings come from field work in Portugal and laboratory studies based in New Zealand (Spider Laboratory of the University of Canterbury) and Portugal. However, the work in Chapter 7 was an exception. This word was undertaken near the end of the time I had available for my thesis work, and during this final period I was situated in Portugal. The work in this chapter required especially large sample sizes and long-term rearing of *C. algerina* and prey spiders, and this could not be achieved in Portugal. However, my supervisor, R.R. Jackson was at the time situated in a laboratory in Kenya (Thomas Odhiambo Campus of the International Centre for Insect Physiology and Ecology, Mbita Point, Kenya). The spacious Kenya laboratory has three full-time experienced technicians who, along with my supervisor, ran the experiments and collected the data on my behalf. The contribution of Godfrey O. Sune, the senior technician

in the Kenya spider laboratory, is especially noteworthy. It was this unique situation that made the work in Chapter 7 possible.

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CHAPTER 2

Cyrba algerina, a jumping spider that lives on the undersides of stones

Abstract

The phenology and the prey records of Sintra and Algarve populations of *Cyrba algerina* in Portugal are described for the first time. The two populations had similar phenology, with males reaching maturity in April followed by females a few weeks later. May appeared to be the primary mating season. The first spiderlings were found in late July. Spiderlings over wintered as juveniles and reached maturity in the following spring. No dense nests were observed during the winter months in the Sintra population, suggesting that the environmental conditions to which this population is subjected are not as severe as that of a population studied earlier from Azerbaijan, where this type of nest is commonly found. Spiders represented 68% of the prey records in the Sintra population, *Trachyzelotes bardiae* (Gnaphosidae) accounting for 70% of the spider prey. The second most frequent prey species in Sintra were unidentified bristletails (Lepismatidae), accounting for 32% of the prey records. The Algarve population seemed to have a more entomophagic diet compared to that of the Sintra population. However, surveys in Algarve did not provide a sufficient number of prey records to conclude this with certainty.

Introduction

Along with more than 12 other genera, the genus *Cyrba* belongs to the subfamily Spartaeinae. Considered a basal branch of the spider family Salticidae (Maddison & Hedin 2003), Spartaeines are characterised by having primitive morphological features (Wanless 1984a). Behaviourally, the subfamily Spartaeine is unusual; in contrast to most jumping spiders, which are know for being active predators that prey especially on insects, the majority of the spartaeines studied to date are also araneophagic predators (i.e., they prey on other spiders). Besides taking spiders and insects as prey, various spartaeines are also known to eat other spiders' eggs, and practise kleptoparisitism, by entering other spider's webs and then rob them of their insect prey (Jackson & Blest 1982, Jackson & Hallas 1986a,b, Jackson 1990a, 2002).

Cyrba algerina Lucas is the most widely distributed species in the subfamily Spartaeinae, and the only one with a wide distribution outside the tropics. Being found primarily in xeric

habitats, stretching from the Canary Islands through the Mediterranean Region and into Central Asia, this species has the widest geographic distribution known for any spartaeine (Wanless 1984a). Having such a wide geographic distribution, it is reasonable to expect that the prey species available to the different populations of *C. algerina* are also considerably different, each population probably having its own particular diet. Nevertheless, the diet of *C. algerina*'s populations has been studied only once, with this being for a population in Azerbaijan (Guseinov *et al* 2004).

The aim of this Chapter is to provide information on the natural history, habitat and prey records on two *C. algerina*'s populations in Portugal. This is important preliminary work for the later Chapters, as it enabled me to identify species that are potentially relevant to each of the populations.

Methods

Cyrba algerina

C. algerina is a medium-size salticid, with adult males usually being slightly smaller (body length 6-8 mm) than adult females (body length 8-10 mm). Juveniles and adult females are orange-brown and have a vague pattern of spots and chevrons. Adult males are more richly coloured, having an orange cephalothorax and contrasting white patterns on a black abdomen. Legs are black with longitudinal white stripes (Fig. 1) (Wanless 1984b).

Populations

Two populations of *C. algerina* from Portugal were chosen for this study, one from Sintra and one from the Algarve. Both localities have a Mediterranean climate, characterised by hot, dry summers, and cool rainy winters. The Algarve site was located in the Barrocal subregion, in the southeast end of Portugal (37º 8' N, 7º 41' W; 107 m above seal level). Mean annual temperatures in this site vary between 16-17.5 ºC (Pena & Cabral 1992) and mean yearly rainfall is 523 mm (http://www.meteo.pt/pt/clima/info_clima/clima_normais.jsp; accessed 10/03/07). The vegetation is mainly composed of carob trees (*Ceratonia siliqua),* mastic shrubs (*Pistacia lentiscus*), Holm oaks (*Quercus rotundifolia*), kermes oaks (*Quercus coccifera*), Mediterranean fan palms (*Chamaerops humilis*), rockroses (*Cistus albidus, C. crispus, C. monspeliensis*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula stoechas*), *Sedum,* sp., short ephemeral grasses and bulbs (Fig.2).

Figure 1. *Cyrba algerina* juvenile (a), subadult male (b) female (c) and male (d) showing typical species coloration.

The Sintra site (38º 51' N, 9º 20' W; 60 m above sea level) was located at a linear distance of about 240 Km northwest of the Algarve location. Mean annual temperatures in this site vary between 12.5-15ºC (Pena & Cabral 1991) and mean yearly rainfall is 751 mm (http://www.meteo.pt/pt/clima/info_clima/clima_normais.jsp; accessed 10/03/07). Vegetation was mainly composed of olive trees (*Olea europaea*), kermes oaks (*Quercus coccifera*), common ash (*Fraxinus excelsior*), myrtle (*Myrtus communis*), spurge flax (*Daphne gnidium*), evergreen rose (*Rosa sempervirens*), blackberry (*Rubus ulmifolius*), common smilax (*Smilax aspera*), asphodel (*Asphodelus* sp.), honeysuckle (*Lonicera peryclymenun*), mayweed chamomile (*Anthemis* cotula), lesser periwinkle (*Vinca minor*), lesser celandine (*Ranunculus ficaria*) and short ephemeral grasses and bulbs (Fig. 2).

Field surveys

Fieldwork was carried in Sintra and Algarve during spring and early summer between 2002 and 2006. The Sintra location was also surveyed once a month during the autumn and winter of 2006. All surveys were carried between 1100 and 1800 h. Logistic constraints meant that the Algarve location was surveyed only about once a month (c. 30 hours), whereas the Sintra location was surveyed once a week (c. 64 hours).

Surveys were made by overturning each stone encountered in the field sites. Both the undersides of the stones and the ground beneath them were examined for *C. algerina* individuals and potential prey items. The sex and age class of all individuals was determined. Four age classes were recognised: 1) small juveniles (<3 mm in body length), 2) subadult males (individuals with dull-brown coloration and enlarged palps), 3) adult males (individuals with bright orange coloration), and 4) adult females (individuals >3 mm in body length without enlarged palps) (Fig. 1). Any individual holding prey in its chelicerae was placed with the prey in a vial and the prey was then identified.

Adult male and female individuals from both populations were collected and taken to the laboratory to establish cultures. These individuals were used for body-size comparisons. The following measurements of carapace dimensions were taken from individuals as soon as they died: 1) diameter of the anterior median eyes (AME), 2) carapace width at its widest point (CW), and 3) carapace length (CL). Using a binocular microscope at 25x magnification, measurements were taken up to the nearest 37 μm, using an eyepiece micrometer (calibrated with a slide micrometer). Only 49 females provided measurements, as spider bodies tend to decay very quickly in the laboratory. As males tend to get eaten by females before and after mating, I was unable to get measurements from a sufficient number of males.

Figure 2. Algarve (top of page) and Sintra (bottom) sites.

Qualitative assessment of potential prey species

All species encountered during fieldwork, known to be taken by *C. algerina* and other salticids as prey and not much bigger (c. 1 ½ body lengths) than *C. algerina* individuals, were considered as potential prey.

Data analysis

Data were analysed using chi-square tests for goodness of fit and Fisher exact tests (Sokal & Rohlf 1995). Data are presented as mean ± SD.

Results and Discussion

Habitat

C. algerina was usually found in clearings with very rocky ground and low vegetation cover in both locations. When found, *C. algerina* was always on the sides and on the undersides of loose or partly buried stones in close contact with the ground (Fig. 3). *C. algerina* was never found under big piles of stones. Stone size varied from c. 10 cm up to 60 cm (on its longest side). When found, *C. algerina* was usually motionless, occupying a small crevice on the underside of the stone. As many as six *C. algerina* individuals from both sexes and varying age classes were found sharing the same stone, especially under large stones (i.e., larger than 40 cm x 20 cm).

When stones were overturned, *C. algerina* usually stood briefly. Then it usually ran very rapidly for a few centimetres towards the edge of the stone, and disappeared under it. When the stone was turned again *C. algerina* was usually standing in another crevice. *C. algerina* almost never abandoned a stone, even if the stone was successively turned over.

Nests

The Azerbaijan population of *C. algerina* is known to spin two types of nests (Guseinov *et al* 2004). “Sparse nests” are transparent, very sparsely woven silk structures, consisting in only a few crossed strands of silk over a denser silk platform (Fig. 4). “Dense nests” are usually more opaque papery-like structures, similar to those of typical salticids. Dense nests were only found during winter months. During the remainder of the year, only sparse nests were observed in Azerbaijan.

Only the Sintra location was surveyed during autumn and winter months but no dense nests were ever found (i.e., in Sintra, only sparse nests were observed during autumn and winter months). During the rest of the year Sintra and Algarve *C. algerina* were often found inside sparse nests.

Figure 3. *Cyrba algerina*'s typical microhabitat

Figure 4. *Cyrba algerina* inside sparse nests on the underside of stones.

Eggsacs

*C. algerina'*s eggsacs consisted on a sheet of dense silk laid against a rock crevice. The eggs were laid in the middle of this dense sheet and covered with a second thin layer of silk with distinctive clusters of white spots, small tuffs of very densely woven silk embedded on the outer layer of silk (Jackson & Hallas 1986b, Jackson 1990a). Eggsacs were usually found in small crevices on the undersides of stones, but some were also found directly on the soil under overturned stones. Eggsacs were never found under stones smaller than c. 30 cm long. *C. algerina* was usually standing on top of the eggsac, but unattended eggsacs were also found. Whether the eggs were in fact left unattended, or whether this was a simply a consequence of stone overturning is not known. Eggsacs from both populations were similar in appearance.

Phenology

Adult males were usually found by mid April. Adult females were usually found a few weeks later. Adults from both sexes were common until mid May, with slight variations depending on the years. May appeared to be the primary mating season. A decline in the number of males in mid May was usually followed by a decline in the number of females a few weeks later. Eggsacs were usually found in late May, with the first spiderlings appearing late July, early August. By September larger juveniles were commonly found on the undersides of stones. Spiders over wintered as juveniles and reached maturity on the following spring. There were no discernible differences in the phenology of the two populations. However, because the Algarve location was sampled less frequently, I can not rule out the possibility of there having been minor variations that I did not detect.

Qualitative assessment of potential prey species

Other than *C. algerina*, the most often seen spider species in Sintra was *Oecobius machadoi* (Oecobiidae). Also common were three salticids, *Heliophanus cupreus*, *Phelgra* sp., *Menemerus semilimbatus* (Salticidae). *Trachyzelotes bardiae* (Gnaphosidae) was also common. Daddy longlegs spiders (Pholcidae), small orb weavers (Araneidae), *Pardosa* sp. (Lycosidae) and lynx spiders (Oxyopidae) were also present but in lower numbers. Ants (Hymenoptera) were by far the most common insects in Sintra, although bristletails (*Ctenolepisma* sp., (identification still uncertain)) were also common.

The most common spiders in Algarve other than *C. algerina* were other salticids, *Menemerus semilimbatus* being the most common, followed by *Aelurillus* sp. and *Phylaeus chrysops*. Less common spiders were *Palpimanus gibbulus* (Palpimanidae) and some

unidentified species of gnaphosids. The insect fauna in Algarve was similar to that found in the Sintra. In general terms, prey diversity and abundance seemed relatively lower in the Algarve than in Sintra.

Prey records

Although *C. algerina* individuals were commonly found in the field, instances of individuals feeding in the field are rare; only 22 *C. algerina* from Sintra and four from the Algarve were feeding when found. The prey records given here represent the diet of juvenile and female individuals only, as no males were ever found feeding.

The Algarve and Sintra populations of *C. algerina* only took insects and spiders as prey. Although spiders were the most common prey in the natural diet of Sintra *C. algerina*, accounting for 68 % of the prey records (15 out of 22) (Fig. 5; Table 1), the prey records of the Sintra population did not significantly differ in terms of the number spiders and insects taken (χ^2=2.91, NS, N=22). Of the spiders taken, the vast majority were found to be gnaphosids, more precisely, *Trachyzelotes bardiae,* accounting for 32 % of the total prey records, and 70% of the identifiable spiders captured by *C. algerina* (7 out of 10). The remaining three identifiable spiders were one conspecific subadult male, one conspecific adult male, and one zodarid, *Zodarion* sp.

Insects comprised 32% (7 out of 22) of *C. algerina*'s prey records in Sintra, bristletails accounting for 86% of all the insects captured (6 out of 7) and about 27% of all the prey captured by *C. algerina* individuals from this population. The only other insect *C. algerina* was found feeding was a dipteran.

Insects were the most common prey items in the diet of Algarve individuals. Two bristletails (Thysanura) and one cricket (Orthoptera) accounted for 75% (3 out of 4) of this population's prey records. The only spider the Algarve *C. algerina* was found feeding on was a conspecific male (Fig. 1; Table 1). Although the Algarve population seems to have a more entomophagous diet than the Sintra population, when the prey records of the two populations are compared in terms of insect and spider prey taken, the two populations are not significantly different (Fisher exact test P=0.1284, NS, N=26). However, given the low number of prey records obtained for the Algarve population, it is premature to draw any conclusions.

Body size

The mean diameter of Sintra female's anterior median eyes (523 ± 45.40 μm, N=23) was significantly different from that of Algarve females (502 ± 80.25 μm, N=26) (t=2.19, P<0.05;

N=49), Sintra females usually having relatively larger diameter eyes (about 4% bigger) than Algarve females. Carapace width was also significantly different between the two populations (t=2.71, P<0.05; N=49), Sintra females showing relatively wider (about 6% bigger) carapaces (1740 ± 110.62 μm, N=23) than Algarve females (1661 ± 80.25 μm, N=29). The same was true for carapace length, Sintra females showing relatively longer (about 5% longer) carapaces than Algarve females (2481 ± 175.63 μm, N=26 and 2330 ± 118.47 μm, N=23, respectively) (t=3.30, P<0.005; N=49). The ratio of carapace width and anterior median eye diameter was not significantly different between the two populations (t=0.56, NS; N=49).

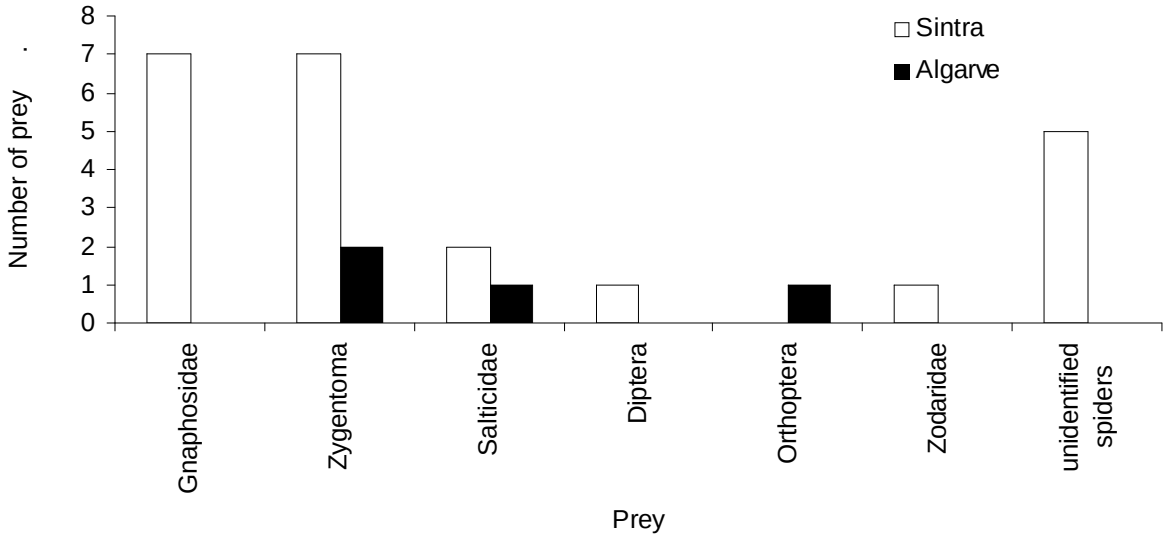

Figure 5. Prey records for *Cyrba algerina* from Sintra (N=22) and Algarve (N=4) populations in Portugal.

Table 1. Prey records for *Cyrba algerina* from the Algarve and Sintra populations.

Order		Algarve	Sintra
Araneae			
1.	Gnaphosidae: *Trachyzelotes bardiae*		7
2.	Salticidae		
	Cyrba algerina subadult male		1
	Cyrba algerina male	1	1
3.	Zodariidae: *Zodarion* sp.		1
4.	Unidentified spiders		5
Diptera			
1.	Unidentified		1
Homoptera			
1.	Cicadellidae (leafhopper)	1	
Zygentoma			
1.	*Ctenolepisma* sp. (bristletails)*	2	6
Total		4	22

* Identification still uncertain.

General Discussion

Most jumping spiders are diurnal, cursorial predators that actively capture their insect prey out in the open by stalking, rather like a cat stalking a mouse (Land 1974). It is usually during these activity periods that salticids are seen wandering around on walls, on top of stones and tree trunks.

C. algerina is unusual in this respect. During the entire duration of the fieldwork *C. algerina* was never seen out in the open; this species was always on the undersides of stones when found. Although this does not prove that this species' activity is restricted to this particular microhabitat, it suggests that *C. algerina* excursions away from the undersides of stones are probably infrequent. Alternatively, this species might venture in the open at later hours of the day. Given that most of the fieldwork was done in the morning and afternoons (surveys were never carried after 19:00 h), additional fieldwork at later hours in the day would be necessary to explore this hypothesis.

Although using silk for web building is not part of the repertoire of most salticid species, the majority of jumping spiders builds silk nests. Typical salticid nests are densely woven, tubular structures, with an opening ('door') at each end, and not much larger than the spider itself (Richman & Jackson 1992), providing the salticid with shelter during periods of inactivity (e.g., at night, and when moulting, mating and ovipositing) (Jackson 1979). Besides providing shelter, nests can also protect the resident salticid and its eggs against predators by acting as a physical barrier between the occupants and the predator (Jackson 1976).

Spartaeines are unusual when compared to most salticids, as most of the spartaeine species studied to date do not build dense tubular nests (Jackson & Hallas 1986a, Jackson 1990a-d). Similarly to most spartaeines, the nests built by the Algarve and Sintra *C. algerina* were very sparsely spun, consisting in only a few crossed strands of silk. Given its fragile structure, *C. algerina*'s nests would appear unlikely to provide the spider, or its eggs, with great protection from predators. In fact, in the spring of 2005 mites seemed to be responsible for the destruction of most *C. algerina*'s eggsacs in Sintra.

Dense nests, similar to those built by *C. algerina* individuals in Baku during the winter months (Guseinov *et al* 2004), were never found in Sintra (only Sintra was surveyed during the winter months). The absence of dense nests in Sintra could be related with the different environmental conditions experienced by the two populations. Although both locations are in similar latitudes, winter in Baku is more severe than in Sintra. The fact that most *C. algerina* from Baku where quiescent and inside their nests when found, also suggests that individuals from this population spend the winter months sheltering inside their nests. If such is the case, a

dense, stronger and more resistant nest should be advantageous, as sparse nests are very fragile, and therefore, easily destroyed.

C. algerina's eggsacs were always found on the undersides of especially big stones, and some eggsacs were even oviposited directly on the soil under these stones. Although the stone surfaces exposed to the sun may reach very high temperatures during the day in both locations, the undersides of bigger stones are never very hot to touch, and the soil underneath the stones is often quite moist. By laying their eggs only under large stones, either directly on the ground or on its underside, *C. algerina* may be avoiding the high temperatures that would apply closer to the surface, and potentially protect its eggs against desiccation.

Sintra and Algarve populations were somewhat different in terms of type and abundance of prey available. The most striking difference between the two populations in terms of type of prey available is probably the absence of *O. machadoi* and *T. bardiae* in the Algarve site during field surveys. In general, the Algarve site seemed to have a much lower diversity and abundance of prey; besides other salticids species and bristletails, no other species was present in great numbers in Algarve.

An earlier study (Guseinov *et al* 2004) on the Azerbaijan population of *C. algerina* showed that individuals from this population prey mainly on spiders, supplementing their diet with a wide variety of other arthopods. Compared to *P. fimbriata*, the only other spartaeine for which prey records are available (Jackson & Blest 1982, Clark & Jackson 2000), *C. algerina* from Azerbaijan appears to have a more euryophagic diet (wide diet). The same can be said about the Sintra and the Algarve populations of *C. algerina*. Although spiders, especially *T. bardiae* (Gnaphosidae), were common in *C. algerina*'s natural diet in Sintra, bristletails were also an important part of the diet. In spite of their abundance, *C. algerina* was never found feeding on *O. machadoi* in the field. Its ubiquity in *C. algerina*'s microhabitat in Sintra, as well as the fact that it belongs to the same genus as the most frequent prey of the Azerbaijan population of *C. algerina* make this finding surprising. Its absence in *C. algerina*'s prey records should not, however, be taken as evidence that the Sintra population does not prey on *O. machadoi*. Oecobiids are very small spiders, about 2.5 mm in body length, and are probably rapidly discarded by *C. algerina* after feeding. This, together with fact that spiders are very rarely found feeding in the field, might explain the absence of *O. machadoi* from this population's prey records.

The prey records suggest that the Algarve population might have a diet that is more entomophagic than the diet of the Sintra population but, when compared, the diets of the two populations were not significantly different in terms of the numbers of spider and insect prey

taken. However, given the low number of prey records for the Algarve population, caution should be taken when interpreting the results. Additional fieldwork in the Algarve is necessary in order to reach a more definitive conclusion.

The frequency with which *C. algerina* females are found feeding on males, both in the field and in the laboratory, suggest that cannibalism by females is particularly common in *C. algerina*. Although the function of sexual cannibalism remains the subject of debate (Johns & Maxwell 1997, Prenter *et al* 2006), several hypotheses have been suggested to explain its maintenance and evolution among arthropods. Besides the foraging strategy hypothesis (i.e., male cannibalism as a source of nutrient diversity) (Johnson 2001), sexual cannibalism has also been considered a female mate choice mechanism (i.e., females choosing to cannibalise smaller males instead of mating with them), a case of mistaken identity, (see Prenter *et al* 2006 for a review), a consequence of female unreceptivity to mating (Jackson & Hallas 1986a), the result of male sacrifice (i.e., a mechanism to increase copulation duration so as to increase the number of fertilised eggs) (Andrade 1996), or simply a consequence of female voracity (Fromhage *et al* 2003). However, as the predatory sequences leading to the male's death were never observed, there is currently little basis on which to decide which of these hypotheses might better explain sexual cannibalism by *C. algerina* females.

Sintra females were considerably bigger than Algarve females. Whether this is a consequence of prey availability or other environmental conditions versus a consequence of genetic divergence between the two populations will be explored in the following Chapter.

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CHAPTER 3

Geographic variation in the life cycle of *Cyrba algerina*'s populations

Abstract

The life cycle of Sintra and Algarve populations of *Cyrba algerina* in the laboratory is described. Sintra and Algarve populations have similar life cycles except for the duration of first and second instars. Males from both populations reach maturity at instar four. Females undergo an additional moult, reaching maturity as fifth instars in both populations. Measurements of the exoskeletons were used to compare body sizes of spiderlings from the two populations. Carapace width and carapace length were highly correlated with anterior median (AM) eye size, both in Sintra and Algarve individuals. *C. algerina* from Sintra have larger anterior median eyes, and wider carapaces compared to Algarve individuals, in all instars. Relative AM eye diameter is, however, similar in the two populations, indicating that laboratory reared *C. algerina* from Sintra are relatively bigger than Algarve individuals. Findings suggest that the differences in body size between the two populations have a genetic basis, however, given that *C. algerina* individuals were produced from field-collected individuals, maternal effects cannot be excluded.

Introduction

Results from Chapter 2 showed that adult *Cyrba algerina* females from Sintra tend to be larger than *C. algerina* Algarve females. In order to determine the level to which the observed variation in body size is under genetic control, *C. algerina* individuals from the two populations were reared in the laboratory from egg to maturity under identical laboratory conditions. By doing this it is possible to rule out the effect environmental differences between the two populations (e.g., prey availability, temperature and precipitation) might have in the body size of *C. algerina*'s Sintra and Algarve individuals. Details on *C. algerina*'s life cycle and development in the laboratory are also provided.

Methods

Rearing

Spiders from the Algarve and Sintra populations were collected in late spring and taken to the laboratory to establish cultures. Maintenance, rearing-cage design and terminology follow those of earlier studies (Jackson & Hallas 1986). Only modifications and critical details are given here.

C. algerina individuals were kept in individual cages. Each cage contained a piece of dark cardboard folded in a harmonium shape. The cardboard was kept in place inside the cage by a thin bamboo stick pierced through the cardboard. This provided the spider with darker recesses in which it could build its nest and spend periods of inactivity and oviposit, while providing some environmental enrichment, shown to be important for a healthy development and maintenance of salticids in the laboratory (Carducci & Jakob 2000). Spiders were kept under a 12 h/ 12 h dark/light regime at 25ºC and 60% humidity. Adult spiders were fed every 5-7 days on a mixed diet of fruit flies (*Drosophila melanogaster*), juvenile New Zealand nursery-web spiders (*Dolomedes minor*) and juvenile crickets (*Gryllus* sp.).

Mating was encouraged by introducing a male *C. algerina* in a cage containing a female. Spiders were left undisturbed for a period of 24 h, after which the male was returned to its own cage. Soon after mating, females usually oviposited in the recesses offered by the folded cardboard. Female cages were inspected for eggsacs twice a week. Eggsacs were then separated from each female *C. algerina*, and the cardboard surrounding the eggsac carefully removed leaving only the necessary amount of cardboard to keep the eeggsac in place. The remaining cardboard was cleaned with a dry paper towel and placed in a separate clean cage. This ensured a higher hatching success, as the eggs tended to be destroyed by mites (possibly carried by the fruit flies used to feed *C. algerina*) when left in the same cage as the female.

As soon as spiderlings dispersed they were transferred to petri dishes (85 mm in diameter). A shaded environment was created by covering half of the petri dish with a half circle of black cardboard. This provided spiderlings some shelter from full light, simulating the natural light conditions found in this species' habitat. Several strips of folded cardboard were placed inside each petri dish to increase environmental complexity and create additional nesting areas. Water was provided through a soaked cotton wick placed on the side of the petri dish.

C. algerina spiderlings shared the petri dish with all their siblings until they reached the third instar (see below for definition). Siblings were then divided into smaller groups (of two siblings each) and moved to bigger cages (120 X 60 mm). The reason for rearing spiderlings with their siblings (as opposed to rearing them in separate cages) was that *C. algerina* spiderlings reared in isolation in the previous year were less responsive in general and specifically to prey. A

similar effect has been shown in lycosid spiderlings (Punzo & Ludwig 2002), in which early contact with the mother and siblings was of vital importance to the development of the spider's central nervous system, negatively influencing the capacity for spatial learning, as well as the ability to capture prey. Although rearing siblings in groups leads to a reduction in the numbers of spiders reaching maturity (due to frequent cannibalism among siblings), the benefits seem to over ride the costs.

First instar spiderlings were fed whiteflies (Aleyrodidae) and sugar water prepared with sucrose. Sugar water was provided through a soaked cotton wick placed on the side of the petri dish. Second instar spiderlings were fed a mixed diet of whiteflies, fruit flies, and nursery web spiderlings. All other instars were fed a mixed diet of fruit flies, nursery-web spider juveniles, wax worms (*Galleria mellonella*) and small crickets (body lengths 0.50 to 0.75 relatively to that of *C. algerina's*).

Measurements of body size

To grow spiders must moult repeatedly during their lives. During moulting the old exoskeleton is discarded and replaced by a new and bigger exoskeleton. Immediately after moulting the new exoskeleton can be stretched to a certain point, and accommodate a larger body, until the next moulting event (Foelix 1996). Body size measurements taken from spider exoskeletons can therefore be useful indicators of spider instar (Jackson 1978, Hallas 1989), given that unlike the spider's abdomen, which can extend after a big meal or if the female is gravid, the spider's exoskeleton is rigid.

Cages were inspected for exoskeletons twice a week and the following characters were measured: 1) diameter of the anterior median eyes (AME), 2) carapace width at its widest point (CW), and 3) carapace length at its longest point (CL). Measurements were taken up to the nearest 37 μm, using an eyepiece micrometer (calibrated with a slide micrometer) on a binocular microscope at 25 X magnification. In total 554 exoskeletons were measured. Carapace dimensions were used as an indication of spider size.

Terminology

Following earlier studies (Jackson 1978), the stage between the rupturing of the egg and the first true moult was called the postembryo. The following stage after the first moult was the first instar. The following instars were numbered sequentially until they reached the subadult stage (instar preceding maturity). Subadult males are easily recognised by the enlargement of the palps. Recognition of subadult females is, however, more difficult, requiring close examination

of the spider's anterior ventral abdomen. Spiderlings disperse as first instars, the first moult having occurred while inside the nest. After reaching maturity, spiders do not undergo further moulting. Measurements taken from a moult corresponded to spider size from previous instar (i.e., measurements taken from the second moult corresponded to first instar spiderlings and so forth).

Data analysis

Data were analysed using Student t-tests, Wilcoxon signed-rank tests, Mann-Whitney U-tests and linear least-squares regression (Sokal & Rohlf 1995). Data are presented as mean ± SD.

Results

Oviposition

28 *C. algerina* females from Sintra and 25 females from Algarve oviposited in the laboratory. The Sintra and Algarve females laid a total of 78 and 76 eggsacs, respectively, yielding a total of 293 and 262 spiderlings, respectively. Only about half of the eggsacs laid by each population were fertile (38 (49%) eggsacs from Sintra and 39 (51%) from Algarve). Females from both populations laid a similar number of eggsacs (3 ± 1.1 eggsacs, min=1, max=5 for Sintra females, and 3 ± 1.5 eggsacs, min=1, max=8, for Algarve females. In general, only the first two eggsacs laid by the females of both populations were fertile. Exceptions were one female from Sintra that produced four fertile eggsacs, and three females from Algarve that produced three to four fertile eggsacs each. Between successive ovipositions, 23 ± 12.3 days for Sintra females, and 20 ± 9.3 days for Algarve females, elapsed.

Hatching

For this analysis only the females that produced two or more eggsacs were used. The number of spiderlings that emerged from the first eggsac was not significantly different in the two populations (Student *t*-test=1.42, NS; N=23); the first eggsacs laid by Sintra females produced about 10.5 ± 4.1 spiderlings (N=12), compared to 8.5 ± 2.0 spiderlings (N=11) produced by the first eggsacs laid by Algarve females. The number of spiderlings that originated from the second eggsac was, however, significantly different between the two populations (Student *t*-test=2.31, $P<0.05$, N=23). Although the Algarve females produced slightly fewer spiderlings per eggsac (7.6 ± 2.4 spiderlings, N=11), the eggsacs laid by Sintra females suffered a decrease in the number of spiderlings of about 50% (5.2. ± 2.7, N=12). The decrease in the number of spiderlings that emerged from the first to the second eggsac was significantly different only for

the eggsacs laid by the Sintra females (Wilcoxon, P<0.05, N=12). However, when the total number of spiderlings produced per female was considered (the spiderlings from all eggsacs), females from the two populations did not differ significantly in the number of spiderlings they originated (Mann-Whitney, NS, N=23).

When all eggsacs laid by females were considered, the number of spiderlings that emerged from an eggsac was not significantly different in the two populations (Student *t*-test=1.26, NS; N=77); 8 ± 4.0 spiderlings (min=1, max=17) spiderlings emerged from the eggsacs laid by Sintra females, compared to 7 ± 2.8 spiderlings (min=1, max=10) from the eggsacs laid by Algarve females.

Postembryonical development

The data presented here provide only an indication of potential trends, not precise information concerning the duration of each instar and growth of the individual because spiderlings shared its cage with its siblings (i.e., it was not possible to assign a particular moult to a specific individual).

In general, *C. algerina* individuals from both populations had similar life cycles (see Fig. 1). As soon as they hatched postembryos had a light-pink coloration, similar to that of the egg. They remained almost completely immobile and usually remained inside the nest until they moulted for the first time. Spiderlings left the nest as first instars, and immediately started hunting prey. First instar spiderlings were light brown in coloration and were easily recognised by their translucent cephalothoraxes. The spiderlings' moveable eye tubes can be seen through the translucent cuticle.

Dispersal of spiderlings occurred 37 ± 4.8 days (N=36) after oviposition for Sintra spiderlings, and 39 ± 3.6 days (N=39) for Algarve spiderlings. After 40 ± 11.2 days (N=17) Sintra spiderlings moulted for the second time, and reached the second instar. The duration of the first instar was significantly longer for Algarve spiderlings (Mann Whitney, P<0.001, N=45); second instar was reached only after 126 ± 39.4 days (N=28) for Algarve spiderlings. Compared to adults, second instar spiderlings had species typical markings and coloration but lesser hair density around the eyes and rest of the body.

The duration of the second instar was considerably longer for the Sintra than for Algarve spiderlings (Mann Whitney, P<0.001, N=34), being 105 days ± 30.6 days (N=18) for Sintra spiderlings but only 44 ± 13.7 days (N=16) for Algarve spiderlings. Males reached the subadult stage on the third instar, and were easily recognised by their enlarged palps. Subadult females were similar to subadult males, except that they did not have enlarged palps.

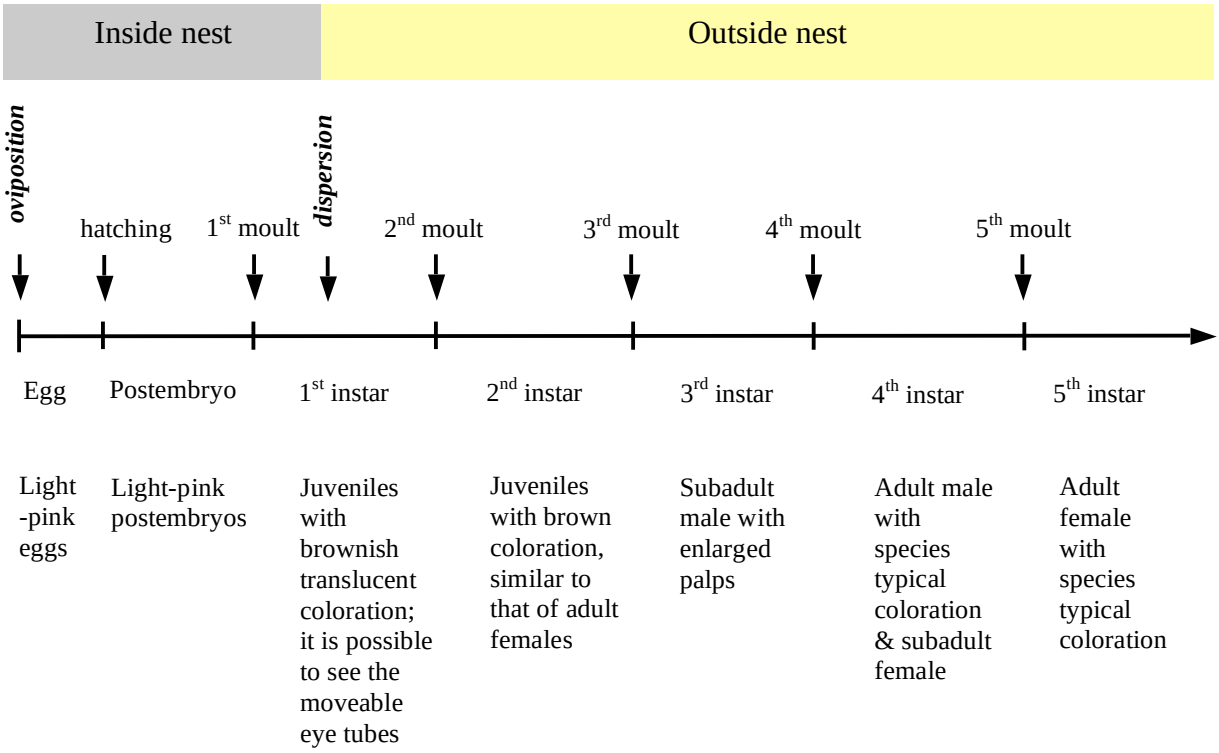

Figure 1. Diagram representing the life cycle of Sintra and Algarve populations of *Cyrba algerina* in the laboratory (not drawn to scale).

The fourth instar was reached after 34 ± 9.0 days (N=13) for Sintra spiderlings, and 27 ± 10.0 days (N=16) for Algarve spiderlings. Males showed the species typical coloration, a bright orange cephalothorax and black abdomen with contrasting white patterns. Upon reaching the subadult stage, females resembled the previous instar, except that they were bigger.

After 26 ± 12.3 days (N=12) for Sintra spiderlings, and 24 ± 2.9 days (N=3) for Algarve spiderlings, females reached maturity as fifth instars (only females underwent this additional moult). Coloration and markings were similar to that of previous instars.

Males reached maturity after 240 ± 12.3 days (N=17) for Sintra males, and 245 ± 12.2 days (N=15) for Algarve males. Sintra and Algarve females reached maturity a couple of weeks later, after 249 ± 8.2 days (N=16), and 257 ± 7.1 days (N=15), respectively. Males reached maturity significantly faster than females in Sintra (Mann Whitney, $P<0.05$, N=33) and in Algarve (Mann Whitney, $P<0.001$, N=30). Sintra females reached maturity faster than Algarve females (Mann Whitney, $P<0.01$, N=31). A similar trend for males was not significant.

Body size

A total of 554 exoskeletons (first to fourth instar) from Sintra and Algarve spiderlings were measured (Table 1). Third instar spiderlings can be easily assigned to either sex through the absence (in juvenile females) or presence (in subadult males) of enlarged palps. This meant that I could compare the exoskeletons from subadult males with the exoskeletons from juvenile females from corresponding moult (third measurable moult). However, as subadult males were not significantly different from juvenile females in any of the parameters measured, data was pooled.

In all instars (Fig. 2), anterior median eye diameters were larger for Sintra spiderlings than for Algarve spiderlings (Table 1); Sintra spiderlings also had wider carapaces than Algarve spiderlings in all instars (Fig. 3 and Table 1). Although Sintra spiderlings form all instars have longer carapaces than Algarve spiderlings (Table 1), this difference was only marginally significant in third instars (Fig. 4).

The coefficients of variation for the all the characters measured were similar, giving no indication that some features are more variable than others. Using the standard deviation around the mean, an interval (mean ± SD) was determined for all instars, for every measured character. There was no interval overlap between successive instars for any of the parameters measured (Table 2).

Table 1. Anterior median eye (AME) diameter, carapace length and carapace width of laboratory-reared *Cyrba algerina* instars from Sintra and Algarve. Measurements were taken from exoskeletons to nearest 37 µm. SD, standard deviation; CV, coefficient of variation.

	1st instar		2nd instar		3rd instar		4th instar	
	Algarve	Sintra	Algarve	Sintra	Algarve	Sintra	Algarve	Sintra
AME diameter								
Mean	223	228	262	270	309	323	378	393
SD	9.25	13.10	19.59	18.21	21.32	25.15	25.67	27.47
Min.	203	203	221	240	258	277	332	351
Max.	240	258	295	314	351	369	406	443
CV	0.04	0.06	0.07	0.07	0.07	0.08	0.07	0.07
N	74	169	38	120	36	77	13	27
Student *t*-test	*t*=3.26; P<0.01		*t*= 2.43; P<0,05		*t*=2.82; P<0.01		*t*=1.69; NS	
Carapace length								
Mean	1041	1041	1233	1247	1468	1525	1769	1839
SD	40.42	78.18	86.38	116.45	118.02	119.10	119.90	139.57
Min.	996	886	1070	1033	1218	1292	1587	1587
Max.	1107	1144	1365	1439	1661	1771	1956	2103
CV	0.04	0.08	0.07	0.09	0.08	0.08	0.07	0.08
N	5	14	19	50	34	72	17	25
Student *t*-test	*t*= 0.01; NS		*t*=0.48; NS		*t*= 2.28; P<0.05		*t*=1.69; NS	
Carapace width								
Mean	768	792	904	930	1051	1101	1257	1318
SD	42.03	43.50	57.31	62.94	71.59	79.60	69.79	87.78
Min.	701	701	812	812	849	959	1144	1181
Max.	867	923	1033	1089	1181	1273	1365	1439
CV	0.05	0.05	0.06	0.07	0.07	0.07	0.06	0.07
N	74	169	38	120	36	77	13	27
Student *t*-test	*t*=3.33; P<0.01		*t*= 2.29; P<0.05		*t*=3.12; P<0.01		*t*=2.15; P<0.05	
Carapace width/AME diameter								
Mean	3.45	3.50	3.46	3.43	3.40	3.41	3.30	3.36
SD	0.13	0.12	0.13	0.11	0.08	0.10	0.09	0.10
Min.	3.17	3.08	3.20	3.25	3.22	3.22	3.18	3.18
Max.	3.67	3.75	3.83	3.73	3.56	3.73	3.56	3.55
N	74	169	38	120	36	77	13	27
Student *t*-test	*t*= 1.89; NS		*t*=0.53; NS		*t*=0.68; NS		*t*=0.63; NS	

When data from all instars was pooled, the correlation coefficient of AM eye diameter with carapace width was slightly stronger (r=0.95 for Sintra and r=0.98 for Algarve; Fig. 5) than with carapace length for both populations (r=0.89 for Sintra and r=0.94 for Algarve; Fig. 6). This might have been an artefact of the tridimensionality of the spiderlings cephalothorax; the front and back ends of the spiderlings cephalothorax are located in different focal planes, making an accurate measurement of its length more difficult.

The mean ratio of carapace width and anterior median eye diameter was not significantly different between instars in the two populations (Table 1), indicating that relative eye size is reasonably constant during instar development in both populations. Pooling data across all instars for each population provided a mean ratio of carapace length and anterior median eye diameter of 3.45 ± 0.12, and 3.43 ± 0.12 for Sintra and Algarve *C. algerina*, respectively. Relative eye size was not significantly different in the two populations (Student *t*-test=1.57, NS, N=554).

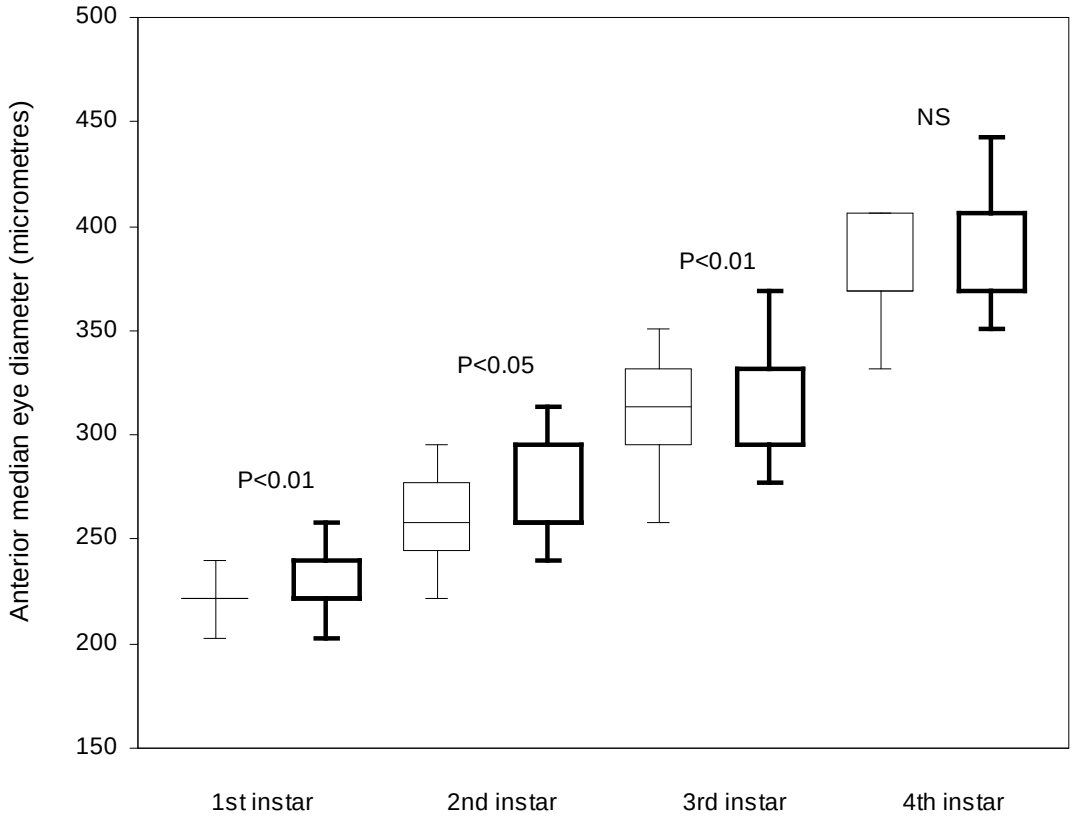

Figure 2. Anterior median eye diameter of *Cyrba algerina* instars from Algarve (thin line) and Sintra (thick line). Measures were taken from exoskeletons. Student *t*-tests (null hypothesis: the means of the two populations are equal) (N=554).

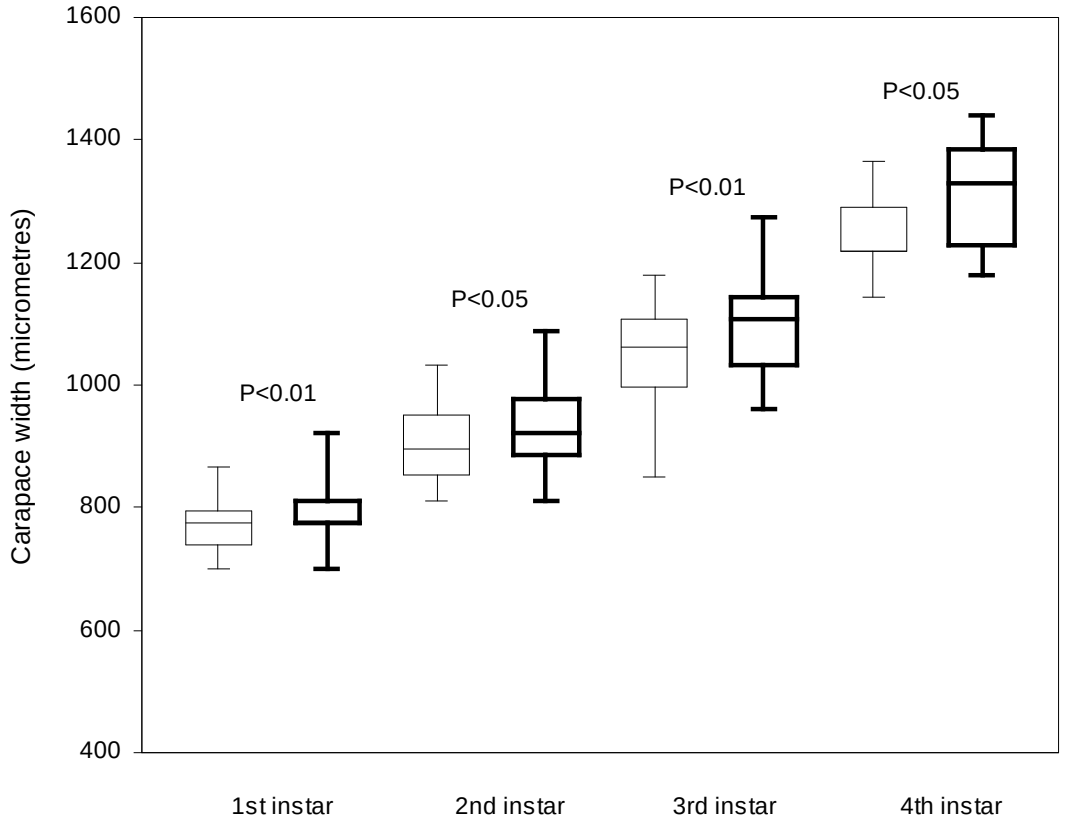

Figure 3. Carapace width of *Cyrba algerina* instars from Algarve (thin line) and Sintra (thick line). Measures were taken from exoskeletons. Student *t*-tests (null hypothesis: the means of the two populations are equal) (N=554).

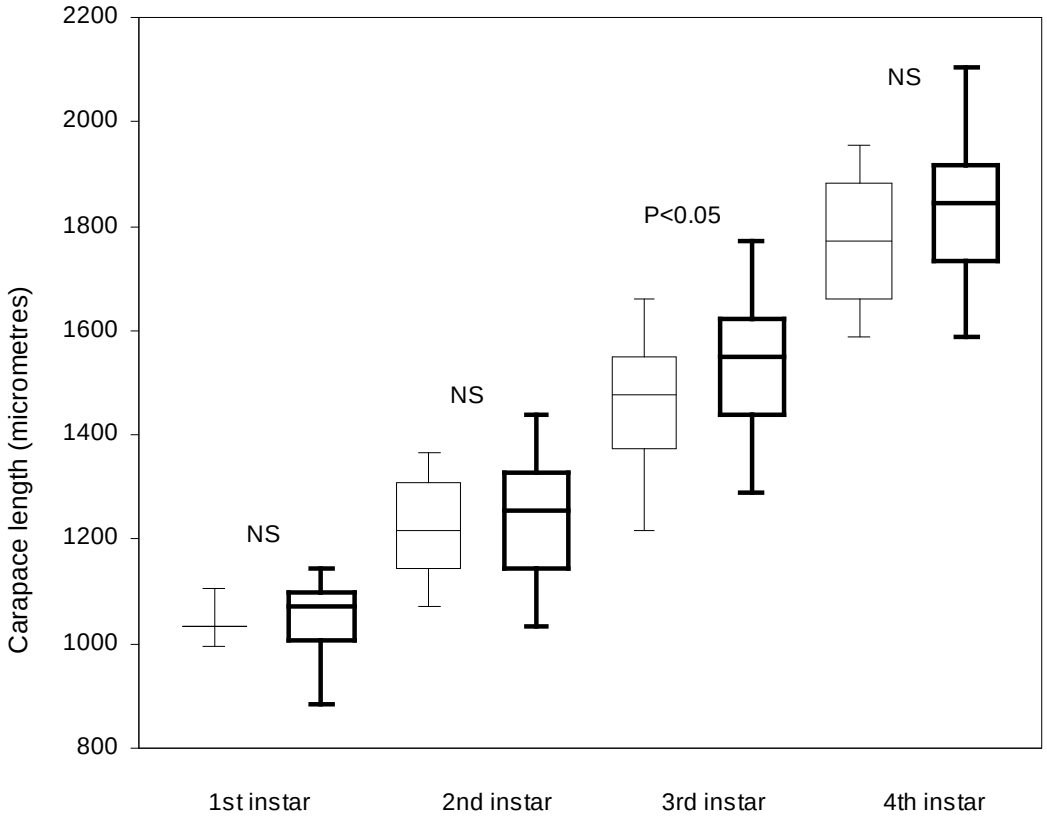

Figure 4. Carapace length of *Cyrba algerina* instars from Algarve (thin line) and Sintra (thick line). Measures were taken from exoskeletons. Student *t*-tests (null hypothesis: the means of the two populations are equal) (N=236).

Table 2. Mean size interval (mean ± SD) for the anterior median eye (AME) diameter, carapace length and carapace width of laboratory-reared *Cyrba algerina* instars from Sintra and Algarve. Interval was calculated by subtracting and adding the SD to the mean. Sample size given in parentheses.

	1st instar	2nd instar	3rd instar	4th instar
AME diameter				
Algarve	214-232 (74)	242-281 (38)	288-330 (36)	352-403 (13)
Sintra	215-241 (169)	252-288 (120)	298-348 (77)	365-420 (27)
Carapace length				
Algarve	1000-1081 (5)	1147-1320 (19)	1350-1586 (34)	1649-1889 (17)
Sintra	963-1119 (14)	1131-1364 (50)	1406-1644 (72)	1700-1979 (25)
Carapace width				
Algarve	726-810 (74)	846-961 (38)	980-1123 (36)	1188-1327 (13)
Sintra	748-835 (169)	867-993 (120)	1022-1181 (77)	1230-1405 (27)

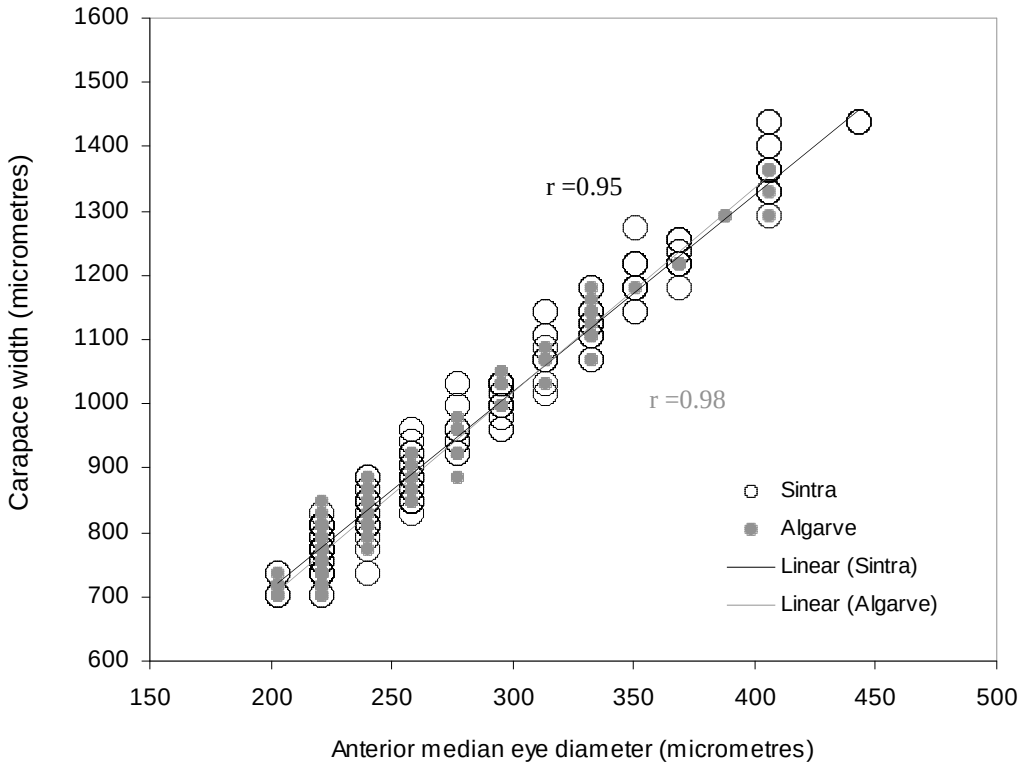

Figure 5. Diameter of anterior median eyes of *Cyrba algerina* from Algarve (N=161) and Sintra (N=393) populations in relation to carapace width.

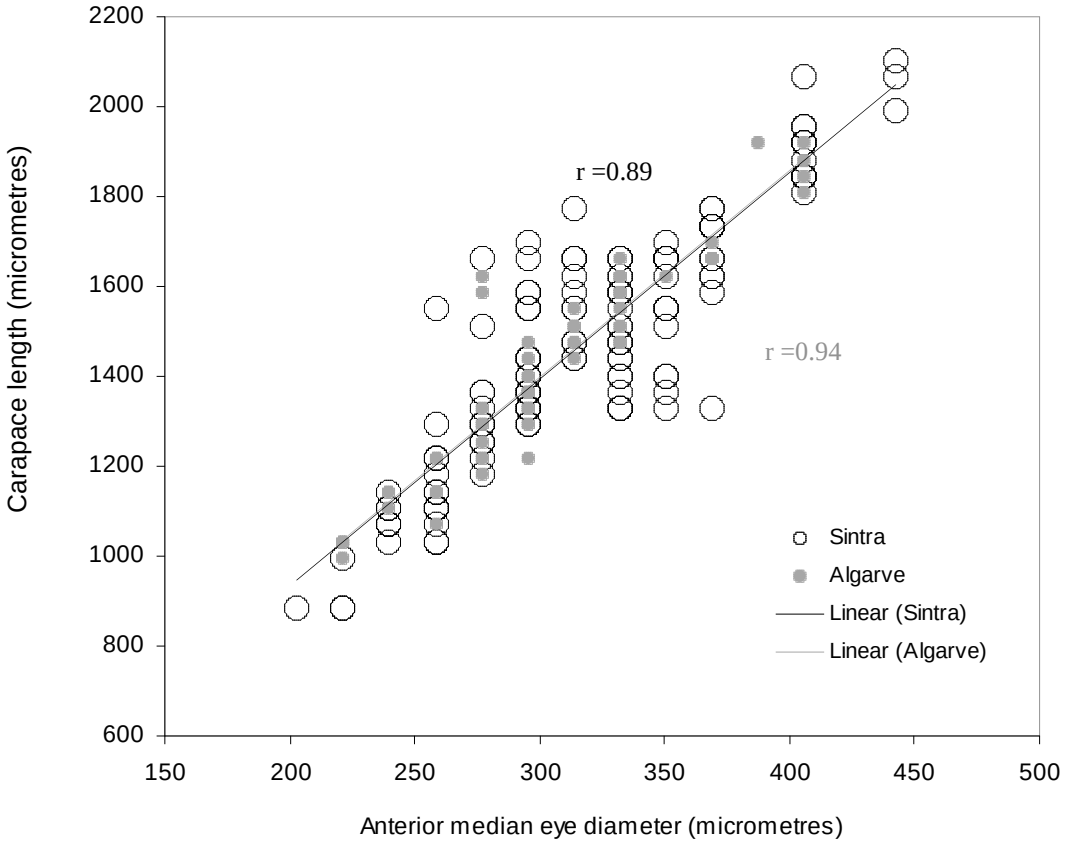

Figure 6. Diameter of anterior median eyes of *Cyrba algerina* from Algarve (N=65) and Sintra (N=171) populations in relation to carapace length.

Discussion

The life cycle of Sintra and Algarve *C. algerina* populations in the laboratory were comparable in most respects. Consistent with findings from other jumping spider studies (Taylor & Peck 1975, Jackson 1978, Matsumoto & Chikuni 1987), *C. algerina* from both populations laid several batches of eggs. Reproducing several times over a lifetime (i.e., iteroparity), as opposed to having a single reproductive event (i.e., semelparity) is expected to be advantageous when juvenile mortality and the risk of complete reproductive failure is high, or when individuals are subjected to fluctuating environmental conditions (Thumm & Mahony 2002). Conversely, semelparity should be favoured when the probability of surviving to reproduce a second time is especially low (Charnov & Schaffer 1973). In Shpak's (2005) words, the iteroparous strategy is akin to that of a gambler that "spreads the risk" and "hedges its bets"; while in a semelparous strategy organisms play a strategy of "all or nothing". Although juvenile and adult mortality has never been studied in detail in this species, field and laboratory observations suggest that egg destruction by predation, cannibalism, could in fact be responsible for considerable juvenile mortality in this species. By spreading their reproductive efforts over multiple batches of eggs *C. algerina* females can potentially reduce the risk of losing its entire progeny.

The total number of spiderlings (i.e., spiderlings resulting from the two batches) produced by *C. algerina* females from both populations was similar. However, when the number of spiderlings that emerged from each batch is considered separately, a decrease in the number of spiderlings over successive batches is observed between the first and the second eggsac laid by the Sintra females. In contrast, the number of Algarve spiderlings that emerged from the first and the second batch of eggs were not significantly different. These results suggest that the two populations have adopted different reproductive strategies. Although the total number of spiderlings produced by Sintra and Algarve females is similar, the Sintra females seem to make a bigger investment in the first batch of eggs followed by a smaller investment in the second batch, whereas the Algarve females seem to make smaller, but similar investments in both batches.

A similar decrease in the number of spiderlings over successive batches has been reported for two other salticid species (Jackson 1978, Matsumoto & Chikuni 1987). According to Jackson (1978) such a decrease could be related with sperm depletion or sperm viability over time. Alternatively, later batches could also contain a greater proportion of "trophic eggs" (i.e., infertile eggs laid by females that provide nourishment to spiderlings) thereby compensating for harsher environmental conditions and lesser availability of prey experienced later in the year. Although either of these two hypotheses suggested by Jackson (1978) could potentially apply to the Sintra females, neither one seems to explain the strategy adopted by the Algarve females.

Different reproductive strategies adopted by the females from the two populations might be related to differences in the availability of prey found at each location. Although actual prey densities were not determined in either of the locations, Sintra appears to have a much greater diversity and abundance of prey when compared to Algarve (see Chapter 2). By making a greater reproductive effort during a spring peak of prey abundance Sintra females are potentially increasing the chances of survival of a great number of its offspring. In the Algarve, given the apparent low availability of prey all year round, a more modest size batch may be optimal, thereby reducing competition for prey among spiderlings, and therefore increasing the number of surviving offspring from each batch.

The most surprising finding from this study is probably concerned with the timing of a long-duration instar. Both populations underwent an instar of considerably longer duration, up to about three to four times longer than the other instars. In Sintra spiderlings the long-duration instar occurred after the spiderlings second moult (corresponding to the second instar). However, in Algarve individuals, the long-duration instar occurred after the spiderlings first moult (corresponding to the first instar). Although currently there is no field data to support it, a possible explanation for the different timing of the long-duration instar in the two populations is an attempt to synchronise the spiderlings development with the different environmental conditions experienced by the Sintra and Algarve populations (e.g., the presence of prey of adequate size).

Consistent with a pattern common in other spiders, *C. algerina* males were smaller than females, matured faster and in fewer moults. Females and males of *C. algerina* both reached maturity in fewer moults than other jumping spiders that have been studied (Taylor & Peck 1974, Edwards 1975 *in* Jackson 1978, Jackson 1978, Matsumoto & Chikuni 1987, Hallas 1989). However, given that *C. algerina* is considerably smaller relatively to the other salticids species studied, the results are not surprising, as the number of moults necessary to reach maturity is usually related with spider size, larger spiders undergoing more moults (Bonnet 1930 *in* Foelix 1996).

Assuming that the relation found between body size and instar for laboratory-reared spiders is similar to that found for spiders growing under natural conditions, the body size measurements taken could potentially be used to determine spider's age. All the measurements taken seem to be good indicators of spider instar, none showing any overlap between instars. However, if working with live spiders, carapace width is probably the best option; besides being the easiest character to measure in a live animal, because the back end of the carapace is partly

obscured by the spider's abdomen, making an accurate measurement of carapace length of living spiders next to impossible.

Field-collected *C. algerina* (Chapter 2) from Sintra were bigger than field-collected individuals of the Algarve, and this same trend was found when *C. algerina* individuals from the two populations were reared under standardised conditions in the laboratory. This suggests that the difference in body size shown by *C. algerina* individuals from the two populations is, at least to a certain extent, a consequence of genetic differences between the two populations. However, because the spiders reared in the laboratory were produced from field-collected females, maternal effects cannot be ignored. Although eggs were separated from females and no maternal care was provided to the eggs, differences between Sintra and Algarve females in terms of maternal provisioning or health during pregnancy might be responsible for the variation in body size between Sintra and Algarve laboratory-reared individuals. Further research is needed to discern the relative contributions of genetic versus environmental influences on between-population size variation in *C. algerina,* including common garden experiments in which Sintra and Algarve individuals are reared in reciprocal locations (see: Schlichting 1986, Relyea 2004).

Interpopulation variation in body size has been reported in another salticid species. Individuals from southern populations of *Phidippus audax* are considerably bigger than individuals from the northern populations. Besides interpopulation differences in body size, there were also interpopulation differences in the embolus and in body markings (Taylor & Peck 1975). Although the differences found between the individuals were not sufficient to ensure reproductive isolation in the laboratory, evidence of at least some level of incompatibility between individuals from the two extremes has led the authors to suggest that the northern and southern forms may represent the two extremes of a clinal population with little interaction between the two extremes occurring.

Morphological differences other than in body size between *C. algerina*'s populations were not investigated. The possibility of additional morphological variation between the two populations should be considered in future research. Although there has been no formal studies of *C. algerina*'s distribution in Portugal, field work during the course of this thesis revealed that this species is commonly found all over the central and southern regions of Portugal. Instead of two isolated populations, the Sintra and Algarve populations may in fact be part of a single much larger population, extending from the south of Portugal to at least the central part of the country.

On the whole, considerable work in necessary on the ecology and behaviour of these populations under natural conditions. This would help to determine the extent to which environmental variation affects the life histories of *C. algerina*'s populations.

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CHAPTER 4

Interpopulation variation in the use of prey-specific attack tactics by *Cyrba algerina*

Abstract

The prey-capture behaviour of two populations of *Cyrba algerina* was investigated in the laboratory. Insect-specific and spider-specific tactics were identified, with attack speed and orientation being the two main differences between the different predatory tactics used by *C. algerina*. Insects were usually approached rapidly and from almost any orientation, whereas spiders were approached more slowly and from specific orientations. Findings indicate that Algarve and Sintra populations of *C. algerina* have evolved prey-specific prey-capture behaviour towards sympatric spider and insect species. Oecobiids, *Trachyzelotes bardiae* and bristletails were the primary target species against which the Sintra *C. algerina* used specialised capture behaviour. In contrast, Algarve *C. algerina* adopted specialised capture behaviour towards bristletails but there was no evidence that individuals from this population adopted specialised capture behaviour against oecobiids or *T. bardiae*. Results suggest that interpopulation variation in the use of specific prey-capture behaviour is related to the prey available to each *C. algerina* population. *C. algerina*'s populations appear to have become locally adapted to local prey.

Introduction

Typical jumping spiders are predators of insects, which they capture using highly developed vision (Jackson & Pollard 1996). During typical predatory sequences the salticid first orients by swivelling its cephalothorax around to bring the principal eyes to bear on the prey. The salticid then aligns its abdomen with its cephalothorax and begins slowly stalking the prey. When close, the salticid pauses, lowers its body and, after fastening a dragline to the substrate, it leaps on the prey (Forster 1982).

However, there are a few salticids for which this description does not apply. The most dramatic examples are found among a primitive salticid subfamily, the Spartaeinae. Renowned for being versatile predators (for definition see: Curio 1976), most spartaeines use a diverse array of specialised predatory tactics, each specific to a particular type of prey or situation; besides being effective cursorial predators of insects, spartaeines are also known to invade alien webs

and perform aggressive mimicry to catch the resident spider and feed on its eggs (Jackson & Hallas 1986a,b, Jackson & Wilcox 1993, Jackson *et al* 1998).

Predatory versatility has been shown to vary geographically in the genus *Portia* (intraspecific geographic variation), geographically separated populations of single species of *Portia* adopting distinctively different innate predatory strategies, with these strategies being adaptively fine-tuned to local prey (Jackson & Hallas 1986a, Jackson & Carter 2001, Jackson *et al* 2002). A well-known example is the specialised behaviour by which *P. fimbriata* from Queensland captures the females of a particular salticid species, *Euryattus* sp., a common species in *P. fimbriata*'s habitat in Queensland. Female *Euryattus* sp. build their nests by suspending a dead, rolled-up leave by silk strands from a tree trunk, a rock ledge or the vegetation. By simulating male *Euryattus* sp. courtship, Queensland *P. fimbriata* lures *Euryattus* sp. females out of its suspended leaf nests, capturing them as they emerge from the safety of the nest. No other population of *P. fimbriata* is known to use this behaviour against this or any other prey (Jackson & Wilcox 1990, 1993).

Similarly to *P. fimbriata*, *Cyrba algerina*, another spartaeine species, is also known to be a versatile predator that adopts different species-specific specialised predatory behaviour against a variety of prey types (Jackson & Hallas 1986b, Jackson 1990). However, until recently (Guseinov *et al* 2004) *C. algerina*'s predatory behaviour had only been examined in the laboratory, and only in tests using the allopatric prey species that were available in the laboratory. Little attention has been given to how this predator responds to sympatric prey. Yet *C. algerina* has a wide geographic range and it is known that different selections of prey are available for *C. algerina* in different localities (Chapter 2). This is the rationale for the hypothesis I consider in this Chapter: that, for *C. algerina*, as for *P. fimbriata*, prey-capture behaviour has become locally adapted to locally abundant prey species. In this Chapter I investigate the predatory strategies adopted by Algarve and Sintra *C. algerina* populations, considering both insects and spiders and both sympatric and allopatric prey.

Methods

Maintenance, rearing-cage design and terminology follow those of earlier studies (Jackson & Hallas 1986a), only modifications and critical details are given here. Animals were kept under a 12-h/12-h dark/light regime, all testing was carried between 0900 and 1800 h. *C. algerina* was fed a mixed diet of fruit flies (*Drosophila melanogaster*) and juvenile New Zealand nursery-web spiders (*Dolomedes minor*) (Pisauridae) every 5-7 days. Hunger level was standardised by keeping each individual of *C. algerina* without prey for 5 days before testing.

The choice of prey was based on the population's prey records and on the most common "potential prey" found during fieldwork in the Algarve and Sintra sites. "Potential prey" were all prey known to be taken by other salticids and not much bigger than *C. algerina* (i.e., less than 1.5X the body length of *C. algerina*). Predatory encounters were staged between *C. algerina* and the following prey species: *Oecobius machadoi* (Oecobiidae), *Trachyzelotes bardiae* (Gnaphosidae), *Pardosa* sp. (Lycosidae), *Heliophanus cupreus, Oxyopes* sp. (Oxyopidae) from Sintra; *Menemerus semilimbatus* (Salticidae), ants (Hymnoptera), and bristletails (Lepismatidae, *Ctenolepisma* sp. (identity still uncertain)) from Sintra and Algarve; house flies (*Musca domestica*) and juvenile New Zealand nursery-web spiders (*D. minor*) from stock cultures.

Because cultures of *O. machadoi* proved to be difficult to maintain in the laboratory, *O. amboseli* (Oecobiidae), a Kenyan species, similar in appearance and body size to *O. machadoi*, was used as a substitute so that adequate sample sizes were possible. No significant differences were found in the behaviour of *C. algerina*'s populations when tested with either oecobiid species, so data was pooled. Apart from *O. amboseli*, fruit flies and house flies, all prey was collected from the field as needed. All tests were carried using live prey. Except for bristletails, all prey used were smaller or similar in size to *C. algerina*.

Predatory encounters were staged in plastic transparent petri dishes (85 mm in diameter and 12.5 mm high). These proved to be suitable arenas, as *C. algerina* is a medium-sized spider and does not usually leap during locomotion (see below). Preliminary observations showed that *C. algerina* has a strong tendency to walk on the sides and edges of petri dishes. Because oecobiids also seem to adopt the sides of petri dishes when building their nests, a specific experimental arena was designed to test *C. algerina* with oecobiids, so as to minimise the frequency with which *C. algerina* contacted the oecobiid's nests merely by chance.

The arena had a flat circular (85 mm in diameter) plastic base. A small plastic disc (20 mm in diameter and 5 mm high), from which about a quarter had been removed, was attached to the base, 15 mm from the side (Fig. 1). The outer edges of this disc were sanded to discourage the oecobiid from building its nest against these. This created a single suitable edge ('artificial crevice') against which the oecobiid could build its nest in the arena. Oecobiids were induced to build a nest against the artificial crevice by surrounding the experimental arena with water, creating an island. About 1 week before testing, oecobiids were put into the artificial island (one per island) to allow enough time for these spiders to build their small nests against the artificial crevice. Before each test the oecobiids's testing arena was covered with the lid of a plastic petri dish. All the remaining prey used was placed inside the petri dish 30 min before testing started.

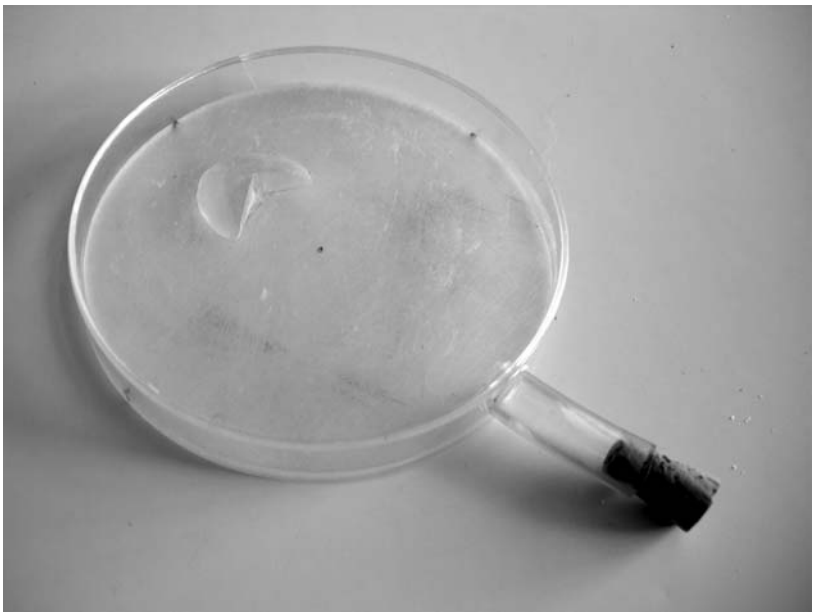

Figure 1. Arena to test *Cyrba algerina* with oecobiids. Oecobiids were induced to build a nest against the artificial crevice by surrounding the experimental arena with water ("artificial island"), leaving the crevice as the only edge available for building the nest.

When staging predatory encounters between bristletails and *C. algerina*, the base of the petri dish was always covered with blotting paper, as the plastic seemed to hinder the bristletails locomotion. The blotting paper was replaced after each test.

Before testing began, an individual of *C. algerina* was taken into a plastic tube (20 mm long and 8 mm in diameter) and its two ends plugged with corks. After a 5-min acclimatisation period, one of the corks was removed and the end of the tube was fit in a hole on the side of the petri dish. *C. algerina* usually walked spontaneously out of the tube and into the petri dish. However, if the test individual was still in the tube after 10 min, the other cork was removed and a soft brush was slowly inserted to entice *C. algerina* out into the petri dish. Testing began when *C. algerina* entered the petri dish. Spiders were observed until captured occurred or until 90 min elapsed, whichever happened first. All predatory encounters were recorded with a video camera to allow posterior viewing and analysis.

The expressions 'usually' or 'often', 'sometimes' or 'occasionally', and 'rarely' were used for frequencies of occurrence of 80% or more, 20-80%, and 20% or less, respectively. The spider's legs were specified as pairs I-IV (anterior to posterior). All *C. algerina* individuals used were collected individuals from the Algarve and Sintra populations. Only *C. algerina* females were used.

As the predatory behaviour of Algarve and Sintra *C. algerina* was similar in many respects, the expression "*C. algerina*" is used whenever a description is applicable for both populations.

Results

ELEMENTS OF BEHAVIOUR

The elements of *C. algerina*'s behaviour are described in earlier works (see Jackson & Hallas 1986, Jackson 1990). Only essential details will be given here.

Crouch

The spider lowered its body, its ventral surface almost touching the substratum.

Erect legs

Legs I and II were fully extended and held parallel to each other c. 45º upward and to the side.

Forward hunched legs

Legs I-III were highly flexed, the femur extended c. 90º to the sides and the patella and tibia were held straight forward (parallel to the spider's body axis). Legs IV were extended and angled straight back. The spider was usually crouching (see above) when it adopted this leg posture.

Lateral hunched legs

The spider's legs I-III were highly flexed (in an arch) and perpendicular to the longitudinal axis of the body. The spider usually crouched (see above) when it hunched its legs.

Leap

A spider leaped by suddenly moving its body forward while rapidly extending legs IV. All legs left the substrate. *C. algerina* only rarely leaped.

Locomotion

C. algerina usually adopted a stop-and-go style of locomotion, moving rapidly forward for a few centimetres, then standing for a few seconds and then moving rapidly again, usually in a different direction. Spiders usually waved its legs in a unique way described in previous work as "swim waving" (see below) and waved its palps up and down (i.e., palp flutter, see below) as they moved around. Palps were usually waved during locomotion but not when standing. *C. algerina* never leaped during normal locomotion.

Lunge

A spider lunged by first lifting legs I and II and extending them forward. The spider then rapidly extended legs IV and suddenly propelled itself forward, returning immediately to its original position. Legs IV did not leave the substrate.

Palp flutter

Spiders fluttered its palps by waving them up and down very rapidly.

Palp plucking

Three modal forms of plucking have been described for *C. algerina*, "up & down", "forward & backward" and "rotary forward & backward" (see Jackson & Hallas 1986b). Each modal form can vary greatly in velocity and amplitude of movement, spiders frequently changing from one modal form of plucking to another.

Probe

Spiders probed by moving their palps backwards and forwards on the silk.

Swim waving

Spiders swim waved by moving their legs I & II together, c. 45º up and to the sides and then, without pausing, bringing them slowly down and inward. Tarsi of both legs usually contacted the substratum on their downward motion. Sometimes only legs I waved.

PREDATORY SEQUENCES

Predation on insects

House flies

C. algerina readily initiated stalking of house flies by approaching very rapidly until about 20 mm from the fly. It then slowed its pace and, when only 1-2 body lengths away, it fastened a dragline to the base or to the side of the arena, raised its forelegs and lunged at the fly from no particular orientation. If the fly was about half of *C. algerina*'s body size, capture usually occurred within less than 30 seconds. *C. algerina* sometimes approached bigger flies, but usually moved away when about 2 body lengths away and rarely captured these flies.

Bristletails

As soon as *C. algerina* detected the presence of a bristletail, it immediately oriented towards it and started chasing the bristletail around the arena very rapidly. When the bristletail stopped, *C. algerina* usually slowed down its pace, crouched and approached it from the side. Slowly moving sideways, *C. algerina* then oriented towards the side of the bristletails' head. When at about one body length away, it hunched its legs forward and stood just beside the bristletails´ head for a few seconds facing it. *C. algerina* then fastened a dragline and lunged at the bristletail's head.

C. algerina usually attacked the bristletail straight from the side (i.e., c. 90º to the bristletails' longitudinal body axis). Other orientations (between 45-90º to the bristletail's longitudinal body axis) were observed less often. *C. algerina* always lunged at the bristletails' head (i.e., it never lunged at the bristletail's mid body or back end, not even when the bristletail was motionless).

After a successful attack the bristletail usually struggled violently for a few minutes, sometimes lifting *C. algerina* in the air, but *C. algerina* never let go of the bristletail. When the

bristletail became motionless, *C. algerina* usually handled it with its forelegs and palps, turning it sideways and grabbing hold of it from underneath. *C. algerina* then carried it to the side of the petri dish and fed on it for several hours.

Whenever the bristletail decamped before an attack or *C. algerina* failed to capture it, *C. algerina* always resumed stalking until capture occurred. Typical predatory sequences were usually a couple of minutes long followed by a single successful lunge. While the bristletail was moving around the arena, *C. algerina* sometimes leaped at it, but usually it failed to capture moving bristletails.

Ants

C. algerina never approached or attempted to capture ants (i.e., it never adopted a predatory posture, and never leapt or lunged) during staged encounters in the laboratory. Whenever approached by an ant, *C. algerina* usually stood still while the ant moved around it. Ants often touched *C. algerina* with their legs and their antennae. When approached by several ants at once, *C. algerina* usually remained calm, but it sometimes moved rapidly away.

Predation on spiders

Oecobiids

With oecobiids, the predatory behaviour adopted by Sintra *C. algerina* was similar to the predatory behaviour adopted by the Baku population of *C. algerina* (Guseinov *et al* 2004).

Oecobiids were usually motionless underneath their nests when encountered. This meant that it usually took some time before it was evident that *C. algerina* had perceived their presence in the arena. After apparently detecting the oecobiid, *C. algerina* slowly approached it, while swim waving and fluttering its palps. When within a couple of body lengths away *C. algerina* usually stopped swim waving, crouched and continued to approach the nest, fluttering its palps, until only a few millimetres away. *C. algerina* then stopped and became quiescent, facing the oecobiid for a few minutes. After the quiescent phase *C. algerina* did one of the following: 1) forward hunched its legs I-III and lunged at the oecobiid; 2) slowly approached the nest and softly plucked the nest's silk with its palps; or 3) slowly moved away from the nest. Most Sintra *C. algerina* individuals drove the oecobiid out of its nest by plucking on the nest's silk, by lunging, or both. Only very rarely did Sintra *C. algerina* approach the nest and simply move away. While plucking on the nest, *C. algerina* usually forward hunched its legs and reoriented towards the oecobiid, slowly moving sideways.

Oecobiids usually fled soon after *C. algerina* lunged or plucked the nest (i.e., oecobiids ran out of their nests). However, if the oecobiid remained in its nest, *C. algerina* continued to probe intermittently, lunged at it, or both. *C. algerina* captured the oecobiid in one of four ways: 1) lunged at the oecobiid and captured it as it left the nest; 2) chased after, overtook and captured the fleeing oecobiid; 3) watched the fleeing oecobiid, stalked it and captured it; or 4) remained quiescent at the nest, and then captured the oecobiid as it returned to the nest. Most often, *C. algerina* captured the oecobiid by lunging at it as it left the nest or after a short chase in the arena. When chasing the oecobiid, *C. algerina* moved very rapidly without swim waving or fluttering its palps. Capture usually occurred within 30 s after a lunge. *C. algerina* sometimes lunged but failed to capture the oecobiid. Whenever this happened, *C. algerina* continued to chase the oecobiid and lunged at it again when within range. On the rare occasions *C. algerina* failed to capture the oecobiid, the reason seemed to be related with fact that oecobiids move very fast, often and suddenly changing direction, after fleeing the nest. C. *algerina* was usually able to track the oecobiid as it moved around the arena but sometimes the oecobiid managed to return to its nest before *C. algerina* could capture it.

During my laboratory study, the Algarve *C. algerina* rarely captured oecobiids. Usually the Algarve *C. algerina* did not approach the oecobiids nest. On the rare occasions it did, it simply walked on top of the nest, apparently without noticing the oecobiid. Walking on top of the nest only rarely provoked the oecobiid to abandon its nest, suggesting that this is not part of a strategy to capture the oecobiid. The Algarve *C. algerina* only rarely lunged or plucked at the nest.

There was a main difference between the Baku and Sintra populations of *C. algerina.* Instead of chasing the oecobiid (the Sintra's *C. algerina*'s usual response), the Baku *C. algerina* usually remained quiescent next to the oecobiid's nest, and captured the oecobiid when it returned to its nest (Guseinov *et al* 2004).

Trachyzelotes bardiae

C. algerina usually approached *T. bardiae* very slowly. When at about 2-3 body lengths away *C. algerina* crouched and adopted an almost imperceptible style locomotion; *C. algerina* moved its legs one at a time. This was done by slowly lifting a leg, moving it forward and placing it down again. Moving a single leg forward could sometimes take several seconds.

While approaching *T. bardiae*, *C. algerina* frequently swim waved and fluttered its palps, and adjusted its orientation so as to be directly head on with *T. bardiae* (i.e., spiders faced each other). This was accomplished by moving sideways very slowly, always keeping *T. bardiae* in its

field of view. When at about one body length away, *C. algerina* usually became motionless, and stood facing *T. bardiae* for a few minutes. After this quiescent phase, the Sintra *C. algerina* usually lunged at *T. bardiae*, the lunge almost always being from directly head on, and grabbed *T. bardiae* by its cephalothorax. Other orientations (c. 45º to *T. bardiae*'s longitudinal body axis), although observed, were rarely adopted. Successful predatory sequences took 10-90 min.

C. algerina did not always succeed at capturing *T. bardiae* and, after a failed lunge, *C. algerina* sometimes approached and attempted to capture *T. bardiae* again. There were rare instances in which *C. algerina* attacked but failed to hold on to *T. bardiae* and both spiders struggled. During a struggle, *T. bardiae* sometimes managed to escape or even kill *C. algerina*, *T. bardiae* never fed on *C. algerina* when this happened.

The Algarve *C. algerina* never approached *T. bardiae* directly head on; approach was usually done from the side, similarly to when approaching cursorial spiders (see below). The Algarve *C. algerina* rarely attacked *T. bardiae*. Most often, Algarve *C. algerina* backed away moving backwards extremely slowly, while facing *T. bardiae*. These *C. algerina* did not usually approach *T. bardiae* again.

T. bardiae sometimes approached *C. algerina* while it moved around in the arena. Whenever this occurred, both the Algarve and Sintra *C. algerina* always moved away very rapidly without ever attempting to capture *T. bardiae*. However, there were rare occasions when both spiders struggled and *T. bardiae* killed *C. algerina.*

Cursorial spiders

C. algerina adopted a similar predatory strategy when capturing *D. minor*, *Pardosa* sp. and *Oxyopes* sp. The expression "cursorial spiders" will be used whenever the predatory sequences described apply equally to all of these species.

C. algerina readily stalked medium-sized cursorial spiders with predatory sequences typically beginning as soon as the prey spider started moving around the arena. *C. algerina* immediately swivelled its body so as to face the spider with its anterior median eyes, and then rapidly approached, stalking it around the petri dish from about 3-4 cm away. When the cursorial spider became motionless, *C. algerina* stopped, crouched and slowly approached it. When about two body lengths away, *C. algerina* pulled its legs I and II back and stood quiescent facing the spider, this quiescent phase usually lasting from a few seconds to a couple of minutes. At this stage, *C. algerina* sometimes reoriented by moving sideways extremely slowly and approached the spider until about one body length away. *C. algerina* usually approached spiders from the side or from the back (between 90 and 180º to the prey's longitudinal body axis), rarely

approaching it head on. Whenever this happened, *C. algerina* slowly moved sideways and eventually came to stand perpendicular to and facing more or less toward the mid-point of the preys' longitudinal body axis. *C. algerina* then hunched its legs I-III forward, faced the spider for a few seconds and lunged, usually stabbing the spider in the cephalothorax. Predatory sequences took between 20 s and 8 min (usually no more than 5 min). After a successful attack, *C. algerina* usually carried the spider under its body to the side of the petri dish and fed for a few hours.

C. algerina rarely failed to capture cursorial spiders after lunging at them. However, when this happened, *C. algerina* immediately resumed stalking. *C. algerina* sometimes lunged at prey spiders even before they became motionless (i.e., it lunged at the spider from the side as it moved around the petri dish). When attacking small spiders (c. ½ of *C. algerina*'s body length), *C. algerina* also tended to approach the prey much faster and to disregard the prey's orientation when lunging; *C. algerina* approached and lunged at small spiders from almost any angle. Although this did not appear to be very relevant when hunting small spiders, it was not a very successful tactic when hunting medium size (similar in size to *C. algerina*) cursorial spiders.

Predatory sequences when prey was motionless were similar in most respects to what has been described above, except that *C. algerina* was slower to react (i.e., to detect the prey's presence in the arena).

Salticids

As no significant differences in the behaviour of *C. algerina* were found during staged predatory encounters with the different salticid species used, the expression "salticid" will be used for all salticid species.

Encounters between *C. algerina* and small juvenile salticids (c. ½ the body length of *C. algerina*) always resulted in very active predatory sequences. In general *C. algerina*'s predatory behaviour when hunting small salticids was similar to when hunting house flies. *C. algerina* quickly approached juvenile salticids as soon as it detected them in the arena. *C. algerina* did not approach salticids from any particular orientation. Juvenile salticids immediately moved away whenever *C. algerina* approached. Capture usually occurred either after a chase or whenever the salticid passed by *C. algerina* within a lunging distance (c. 1.5 cm). Capture was always achieved by lunging and usually occurred within 10 min. *C. algerina* never displayed at juvenile salticids during these encounters.

Interactions with medium size salticids (i.e., similar in size to *C. algerina*) began whenever one of the two salticids (*C. algerina* or the prey salticid) noticed the other's presence

in the arena. Salticids usually approached each other and displayed with erect legs I or I and II, similarly to the threat displays used during intraspecific interactions. Spiders usually displayed for a few seconds after which they usually moved away. When the two salticids faced again they usually displayed at each other again and moved away. *C. algerina* never attempted to capture medium size salticids, nor did any of the salticids tested attempted to capture *C. algerina.*

Discussion

Findings from this Chapter indicate that the Algarve and Sintra populations of *C. algerina* use specialised behaviour to capture sympatric insect and spider prey. *C. algerina* individuals from both populations seem to use the same specific prey capture behaviour when hunting bristletails, a species that frequently occurs in the habitats of both populations of *C. algerina*. However, the Sintra *C. algerina* used a distinct prey-capture tactic when hunting oecobiids and yet another distinct prey-capture tactic when hunting *Trachyzelotes bardiae*, whereas the Algarve *C. algerina* did not use special tactics against these prey. These two prey species, although commonly found in Sintra, are not known to occur in Algarve.

The behaviour used by *C. algerina* from Sintra to capture *O. machadoi* and *O. amboseli* was similar to that used by *C. algerina* individuals from Baku (Azerbaijan) to capture *O. maculatus* (Guseinov *et al* 2004), an oecobiid species sympatric with the Baku population of *C. algerina* but not known to occur in Portugal. The success of *C. algerina*'s predatory strategy seemed to depend on *C. algerina*'s ability to drive the oecobiid out of its nest. Once this was achieved *C. algerina* almost always captured the oecobiid. Although lunging at the oecobiid's nest was also observed, plucking the nest's silk with its palps was the tactic used most often by Sintra *C. algerina* to achieve this.

The use of specialised vibratory web signals to capture web building spiders (i.e., aggressive mimicry) has been described in a few salticid species, including *C. algerina* (Jackson 1990, 2000). An almost unlimited variety of signals can be produced by manipulating (i.e., plucking) web silk with the appendages (i.e., legs, palps) in a variety of forms and combinations. In *C. algerina*'s case, because only the palps are used to manipulate the silk, the number of signals produced is much smaller, its repertoire being only a subset of Portia's (Jackson 1990). Nevertheless, *C. algerina* from Sintra is evidently capable of producing the appropriate vibratory signals to drive the oecobiid out of its nest. The fact that *C. algerina* from Algarve only rarely managed to do so is probably related with the fact that Algarve *C. algerina* almost never approached the oecobiid's nest, and in the few instances in which it did, this behaviour was only rarely used. Although more work is necessary, these observations seem to indicate that the signals produced by Sintra *C. algerina* may be more effective at driving the oecobiid out of its nest than the signals produced by the Algarve individuals, suggesting that *C. algerina* from Sintra might be adaptively fine-tuned to oecobiids as prey.

Except when hunting oecobiids, the major differences between *C. algerina*'s predatory tactics were in lunging orientation and the speed with which *C. algerina* approached prey. In general, speed of approach seemed to be related with the risk involved in the encounter;

especially dangerous prey, such as *T. bardiae*, were usually approached extremely slowly, almost imperceptibly; less dangerous prey, such as insects and cursorial spiders, were approached much faster, and seemingly more carelessly.

When hunting dangerous spider prey, *Portia* spp. are known to carefully adjust its orientation according to the spider species being attacked; *Badumna longinquus* is usually attacked from behind, possibly to avoid this spider's powerful chelicerae, while *Pholcus phalagioides* is attacked from almost any angle, but always through gaps between its long dangerous legs. By carefully adjusting its orientation to specific prey *Portia* spp. seem to have fine-tuned its capture behaviour to match the risks posed by the different spider species (Harland & Jackson 2006). In *C. algerina*'s case orientation did not seem to be important when hunting flies and small salticids, which *C. algerina* usually attacked from almost any orientation. However, when hunting more seemingly dangerous prey, *C. algerina* individuals seemed to carefully adjust its orientation before an attack.

Similarly to *Portia* spp., Sintra *C. algerina* evidently matches its attack orientation to the type of prey it is attempting to capture. Except when *T. bardiae* was the prey, Sintra *C. algerina* almost always attacked cursorial spiders from behind or from the side. However, when hunting *T. bardiae* Sintra *C. algerina* tended to attack it directly head on. *T. bardiae* appeared to be the most dangerous prey used in this study, encounters between the two spiders having, although only rarely, fatal consequences for *C. algerina.* No other prey used ever killed or injured *C. algerina*. The fact that Sintra but not Algarve *C. algerina* used this orientation when attacking *T. bardiae*, and exclusively with this prey, suggests that the tactic used by the Sintra population is specific to *T. bardiae.* Adopting this attack orientation may be related to the risks associated with this prey.

On the contrary, the functional significance of *C. algerina*'s orientation when attacking bristletails is most certainly not related with safety; bristletails are soft-bodied prey and do not seem to pose a threat to *C. algerina*. In the case of bristletails, the adoption of a specific attack orientation by *C. algerina* might be related instead with the bristletails own speed and slipperiness (derived from how the bristletails' body is covered by small scales). The specific attack orientation adopted with bristletails may function primarily in targeting a less slippery, and possibly less protected area of the body (with fewer scales), that would allow a more secure grip as well as a place to insert the fangs.

Even though ants are very common in *C. algerina*'s microhabitat in Algarve and Sintra, *C. algerina* from both populations never attacked ants during staged encounters in the laboratory. This is not surprising, as ants are considered to be dangerous prey to most spiders, with only a

few species among salticids being known to take ants as prey (Li & Jackson 1996, Nelson *et al* 2005a).

Although the target of specialised prey capture behaviour is sometimes a group of prey (e.g., cryptic stalking by *P. fimbriata* seems to target to target salticids in general (see Jackson & Hallas 1986a), cases of remarkable specificity, in which the predator adopts an adaptively fine-tuned behaviour towards a particular species, have also been described in jumping spiders (Jackson & Wilcox 1990, 1993, Clark *et al* 2000, Nelson *et al* 2005b). The most remarkable example might be from recent studies on *Evarcha culicivora.* The smaller instar juveniles of this species adopt prey-specific capture behaviour against a particular genus of mosquitoes, *Anopheles* sp. *E. culicivora* juveniles identify *Anopheles* on the basis of the special posture adopted by mosquitoes from this genus. After ascertaining that the prey is indeed an individual *Anopheles*, the small juvenile *E. culicivora* approaches from behind, and after getting beneath the mosquito's abdomen, attack the mosquito from underneath. *E. culicivora* juveniles are not known to adopt this tactic when preying on any other mosquito genus (Nelson *et al* 2005b).

Whether the prey-capture behaviour the Sintra *C. algerina* uses against *T. bardiae* is specific to this one species or perhaps general to more or less all gnaphosids is unknown. However, the tactic used against oecobiids is apparently not specific to any particular oecobiid species. There was no indication that *C. algerina* behaved differently depending on the particular oecobiid species encountered (sympatric *O. machadoi* and allopatric *O. amboseli*), suggesting that the tactic used by *C. algerina* from Sintra is not targeting a particular oecobiid species. At least three other species of oecobiids are known from Portugal, none of which seem to occur in the habitat of the Sintra or the Algarve population of *C. algerina.* Chances are that that this tactic is wide spread among *C. algerina*'s populations whenever there are oecobiid species sympatric with *C. algerina*.

C. algerina has apparently evolved prey-specific tactics to capture at least some of its most common prey. Moreover, the use of a given tactic seems to be related with the prey locally available to each population. Because all *C. algerina* used were collected from the field, whether *C. algerina*'s behaviour has been shaped by previous experience with these prey is not known. This hypothesis, which seems highly probable, given this species wide distribution, needs to be investigated. Additional work with laboratory-reared individuals deprived of contact with these prey, is necessary in order to investigate this.

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CHAPTER 5

Interpopulation variation in the use of kairomones by *Cyrba algerina*

Abstract

The use of chemical cues from prey was investigated in the Algarve and Sintra populations of *Cyrba algerina*. *C. algerina* individuals from both populations were tested in a Y-shaped olfactometer to assess their response to volatile olfactory cues from sympatric and allopatric prey. Three species of spiders (*Oecobius amboseli*, *O. machadoi*, *Trachyzelotes bardiae*) and one insect species (bristletails) were used as odour sources. When tested with the odour of allopatric prey, there were no significant biases toward choosing prey odour instead of control (no odour source). The Sintra, but not the Algarve, *C. algerina* chose the odour of the sympatric spider prey species (*O. machadoi* and *T. bardiae*) significantly more often than they chose the control, but individuals from neither population chose the odour of the sympatric bristletails. Interpopulation variation in the use of kairomones suggests that *C. algerina* populations are locally adapted to local abundant prey. Relying on olfactory cues from prey during predatory encounters might be especially advantageous for *C. algerina*, a species that lives in a microhabitat subject to low ambient light levels, the undersides of stones.

Introduction

According to their function, infochemicals (i.e., chemical compounds used in chemical communication) are known as "pheromones" when they are used during intraspecific communication and, as "allelochemicals" when they are used during interspecific communication. Allelochemicals can be further subdivided into "allomones" when they benefit the emitter, "kairomones" when they benefit the receiver, and "synomones" when they benefit both the emitter and the receiver (Dicke & Grostal 2001, Schulz 2001).

Although salticids are well known for their remarkably acute vision (Land 1981) and highly elaborate vision-mediated behaviour (Forster 1982, Jackson & Wilcox 1993, Harland *et al* 1999, Jackson *et al* 2002), chemical cues have also been shown to play an important role during both intra- and interspecific interactions; nest and web associated pheromones are known to release male courtship in a few jumping spider species (Pollard *et al* 1987) and, when present in the salticid's draglines, can be used to find females (Taylor 1998) or to assess the fighting ability

of conspecifics (Clark *et al* 1999). In an interspecific context, salticids are also known to make use of chemical cues (kairomones) to locate and identify prey, adopting appropriate behaviour and posture to capture prey even in the absence of optical cues from prey (Clark *et al* 2000a,b, Jackson *et al* 2002).

Cyrba algerina (Salticidae) lives in a very particular microhabitat, the undersides of stones. Given the potentially low ambient light levels found at *C. algerina*'s microhabitat, a strong reliance on olfaction for detection and identification of prey could prove especially advantageous, potentially allowing the spider to detect and identify the presence of a particular prey in advance, as well as allowing the spider to prepare itself for the encounter by adopting specific prey-capture behaviour (Chapter 4).

In this Chapter I investigate the hypothesis that *C. algerina* has become adapted to its particular microhabitat by relying strongly on olfactory cues from prey. Additionally, given that the prey available to the individuals from each population also varies considerably (Chapter 2), I investigate whether the ability to detect and respond to the prey's odour is fine tuned to the specific prey species available to each population (i.e., sympatric prey species).

Methods

General

Maintenance, rearing-cage design and terminology follow those of earlier studies (Jackson & Hallas 1986). Only critical details and modifications of these methods are given here. After collection, animals were kept under a 12-h/12-h dark/light regime (lights on at 0700 h). All testing was carried out between 0900 h and 1800 h. *C. algerina* was fed a mixed diet of fruit flies (*Drosophila melanogaster*) and juvenile New Zealand nursery-web spiders (*Dolomedes minor*) every 5-7 days. Hunger level was standardised by keeping each individual of *C. algerina* without prey for 5 days before testing.

C. algerina individuals were tested in a Y-shaped olfactometer to assess their response to olfactory cues from prey (Fig. 1). Air was pumped into the olfactometer using an aquarium pump. Airflow inside the olfactometer was controlled by two separate flowmeters (Matheson FM-1000 flowmeter) adjusted to 1400 ml/min. Similar airflows have been used in previous experiments. There was no evidence that this airflow impaired *C. algerina*'s locomotion or had any adverse effect in the spider's behaviour inside the olfactometer.

Air flowed from the flowmeters into a stimulus chamber (which contained the prey) and a control chamber (which was empty). Air moved from the stimulus chamber to the stimulus arm and from the control chamber to the control arm, hereafter called "choice arms". From each

choice arm, air then converged into the "test arm" (i.e., the stem of the Y). *C. algerina* was introduced in the apparatus through an "holding chamber" (at the end of the test arm), where it was left for 2 min prior to testing (acclimatisation period). A piece of plastic netting, positioned in a slit between the holding chamber and the test arm, blocked the spider's access to the test arm before the acclimatisation period ended, while allowing the air to flow trough. An opaque barrier between the choice arms and the chambers prevented *C. algerina* from seeing the prey in the stimulus chamber.

Prey providing potential olfactory cues were introduced in the stimulus chamber 30 min before each test. This 30-min period allowed air to circulate evenly and ensured that air pressure was comparable throughout the olfactometer. Tests began when *C. algerina* left the holding chamber and entered the test arm. Time spent in each arm (stimulus versus control arm) was recorded for the following 20 min. The first arm the spider entered was considered its first choice regardless of how long it remained there. A score was obtained for each individual by subtracting the time spent in the stimulus arm from the time spent in the control arm. The arm where *C. algerina* spent more time was considered as the spider's final choice. Tests were aborted whenever a test spider left the holding chamber and simply rushed into one of the choice arms. Whenever this happened, the spider was retested in the following day.

The stimulus chamber was either on the right or left side of the olfactometer, decided at random. The olfactometer was always cleaned between tests with 80% ethanol and then with water, to eliminate draglines or any chemical traces from previously tested spiders. All the *C. algerina* individuals tested were adult females collected from the Sintra and the Algarve populations. No individual *C. algerina* was used more than once in the same experiment.

Blank tests

C. algerina's behaviour inside the olfactometer was assessed in blank tests for possible left-right bias by testing the spiders with empty testing chambers (i.e., no odour source was placed in the stimulus or in the control chamber (i.e., blank tests). Only *C. algerina* females from the Algarve and Sintra populations were used in blank tests.

Odour sources

Three spider species (Oecobiidae: *Oecobius machadoi*, *O. amboseli*; Gnaphosidae: *Trachyzelotes bardiae*), and one insect species (Thysanura: resembles *Ctenolepisma* sp., but identification is currently uncertain) were used as sources of potential olfactory cues.

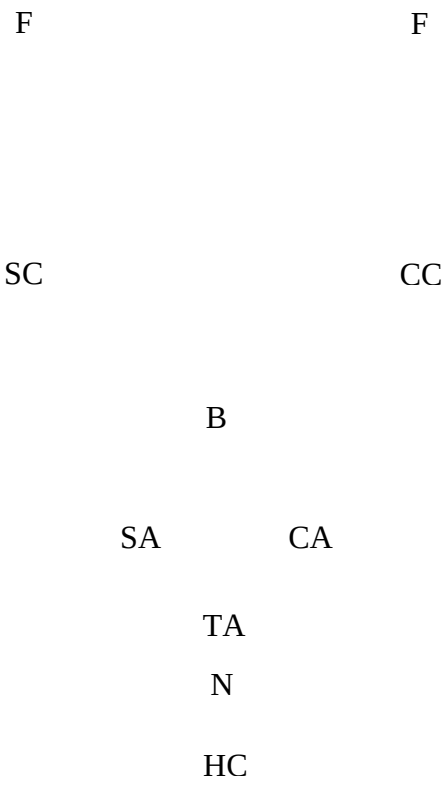

Figure 1. Y-shaped olfactometer (from Jackson *et al* 2002) (not drawn to scale). F - flowmeters, SC - stimulus chamber, CC - control chamber, B- opaque barrier to prevent *Cyrba algerina* from seeing the prey providing potential odour cues, SA - stimulus arm, CA - control arm, TA - test arm, N- net to block *C. algerina* from accessing the test arm during the acclimatisation period, HC - holding chamber.

The choice of these species was based on the prey records of each population as well as on their occurrence in the populations of *C. algerina* studied. *O. machadoi* and *T. bardiae* are known only from Sintra, but bristletails are common in Algarve and Sintra. *O. amboseli* is a Kenyan oecobiid species that is allopatric with both *C. algerina* populations.

Given the small size of all oecobiid species (c. 2.5 mm in body length) compared to the other species used, six oecobiid individuals were placed inside the stimulus chamber as a source of odour. For all other species used, only one individual was used as an odour source. Only female individuals were used as odour sources, with the exception of *O. machadoi*, were both male and female individuals were used as odour sources.

Data analysis

Chi-square tests for goodness of fit were used to analyse the spider's choice, considering separately the first and final choice (null hypothesis for first choice and for final choice: no tendency to choose one arm more often than the other). Scores were analysed using Wilcoxon signed-rank tests (null hypothesis: time spent in one choice arm equal to time spent in other arm). Between-population and between-prey comparisons of the spider's final choice were done using chi-square tests for independence. All statistical procedures were from Sokal & Rohlf (1995).

Results

Blank tests (no odour source present)

Algarve *C. algerina* did not chose the left or the right arm of the olfactometer significantly more often as a first (left=10, right=12; χ^2=0.18, NS, N=22) or as a final choice (left=9, right=13; χ^2=0.93, NS, N=22), nor did the spiders spend significantly more time in either arm (Fig. 2).

The same was true for *C. algerina* individuals from Sintra; spiders did not choose the left or the right arm significantly more often as its first (left =7, right =16; χ^2=3.52, NS, N=23) or as its final choice (left =14, right =9; χ^2=1.09, NS, N=22). Sintra individuals did not spend significantly more time in the left or in the right arm of the olfactometer (Fig. 2).

The final choice of Sintra *C. algerina* was not significantly different from the final choice of the Algarve *C. algerina* (test of independence χ^2=1.79, NS, N=45).

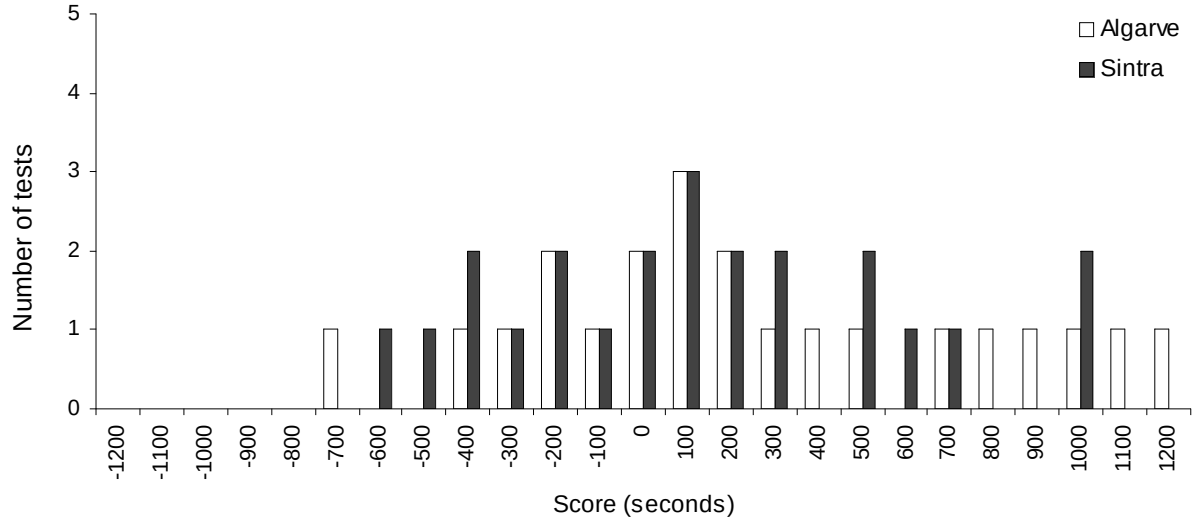

Figure 2. Difference scores from testing *Cyrba algerina* in blank olfactometer tests (no odour source present). Each individual provided a score (time spent on left arm minus time spent on right arm). Algarve (white bars) and Sintra (black bars) *C. algerina* did not spend significantly more time in either of the choice arms (Wilcoxon signed-rank tests: Algarve, P=0.24, NS, N=22; Sintra, P=0.26, NS, N=22).

Testing with odour from sympatric prey species

In general, *C. algerina*'s first choice (arm entered first) was not a good indicator of the spider's final choice (the arm where the spider spent the most time). Except when testing Sintra *C. algerina* individuals with the odour of *T. bardiae*, *C. algerina* showed no evidence of a trend for either of the olfactometer's arms as its first choice (see below).

Sintra population

The final choice of most *C. algerina* individuals from Sintra was the arm containing the odour of female *O. machadoi*. *C. algerina* also spent significantly more time in the arm containing the odour of female *O. machadoi* than in the control arm (Fig. 3). However, *C. algerina* did not choose either arm as its first choice significantly more often than the other (Table 1).

When *O. machadoi* males were used as odour sources, Sintra *C. algerina* did not choose the arm containing the odour of *O. machadoi* males as its first or as its final choice significantly more often than the control arm (Table 1), nor did *C. algerina* spend significantly more time in either arm (Fig. 3). *C. algerina*'s behaviour when tested in the olfactometer with *O. machadoi* males was not significantly different from when tested in the olfactometer with no prey present (i.e., blank tests) (test of independence χ^2=1.43, NS, N=44). Results when testing the Sintra *C. algerina* with the odour of *O. machadoi* females were significantly different from results when testing with *O. machadoi* males (test of independence χ^2=4.16, P<0.05, N=53).

When tested with the odour of *T. bardiae*, *C. algerina* chose the stimulus arm significantly more often as it's first as well as its final choice (Table 1). Spiders also spent significantly more time on the arm containing the odour of *T. bardiae* than on the control arm (Fig. 4). *C. algerina*'s final choice was significantly different from when no odour from prey was present in the olfactometer (i.e., blank tests) (test of independence χ^2=9.71, P<0.01, N=47).

When bristletails were the odour source, Sintra *C. algerina* did not chose either of the arms (stimulus vs control) significantly more often as its first or as its final choice (Table 1), neither was the time spent in each arm significantly different (Fig. 5). *C. algerina*'s final choice when using the odour of bristletails was not significantly different from when no odour was present in the stimulus arm (i.e., blank tests) (test of independence χ^2=1.43, NS, N=44).

Algarve population

C. algerina from Algarve did not spend significantly more time in the arm containing the odour of bristletails than in the control arm (i.e., no odour) (Fig. 5), nor did it chose either arm significantly more often as its first or as its final choice (Table 1). *C. algerina*'s behaviour was not significantly different from when no odour cues from prey were present (blank tests) (test of independence χ^2=0.35, NS, N=42).

Testing with odour from allopatric prey species

When tested with the odour from allopatric prey spider species, neither population of *C. algerina* chose either arm significantly more often as a first or as a final choice (Table 1). *C. algerina* from Sintra did not choose the odour of *O. amboseli* significantly more often as its final choice, nor did the Algarve *C. algerina* choose the odour of *O. machadoi, O. amboseli* or *T. bardiae* significantly more often than the control arm (i.e., the arm containing no odour from prey).

The same applied for the time spent in the choice arms; *C. algerina* individuals from Sintra and Algarve did not spend significantly more time on the arm containing the odour of *O. amboseli* (Fig. 7), *O. machadoi* for Algarve *C. algerina* (Fig.6), or *T. bardiae* for Algarve *C. algerina* (Fig. 4).

Results from testing Sintra *C. algerina* with *O. amboseli* and *O. machadoi* indicate that Sintra *C. algerina* responds to the odour of the two oecobiids species differently (test of independence χ^2=6.65, P<0.01, N=51), approaching *O. machadoi* but not *O. amboseli* on the basis of odour cues alone.

As for Algarve *C. algerina*, its behaviour in the olfactometer when in the presence of the odour of *O. machadoi*, *O. amboseli* or *T. bardiae* was not significantly different from when the olfactometer had no prey (i.e., blank tests) (tests of independence: *O. machadoi* χ^2=1.94, NS, N=50; *O. amboseli* χ^2=0.00, NS, N=46; and *T. bardiae* χ^2=0.40, NS, N=48).

Population comparison

The prey-odour choices of the Sintra and Algarve *C. algerina* were significantly different when the prey was *O. machadoi* (test of independence χ^2=8.93, P<0.01, N=56) (Fig. 6) and *T. bardiae* (test of independence χ^2=6.18, P<0.05, N=50) (Fig. 4).

The behaviour of Sintra and Algarve *C. algerina* was not significantly different when *O. amboseli* (test of independence χ^2=0.02, NS, N=47) (Fig. 7) and bristletails (test of independence χ^2=0.59, NS, N=41) (Fig. 5) were used odour sources.

Table 1. Results from olfactometer tests using sympatric and allopatric spider and insect species as odour sources. Two populations of *Cyrba algerina* were tested: Algarve and Sintra (Portugal). First choice: first arm *C. algerina* entered. Final choice: arm in which *C. algerina* spent most of its time. S - stimulus arm, C - control arm. Chi-square tests of goodness of fit (null hypothesis: spiders choose either choice arm equally often).

	Cyrba algerina	First Choice				Final choice		
Odour source	population	N	S	C	χ^2 goodness of fit	S	C	χ^2 goodness of fit
Oecobius machadoi	Algarve	28	13	15	χ^2=0.14, NS	11	17	χ^2=1.29, NS
Females (Sintra)	Sintra	28	12	16	χ^2=0.57, NS	22	6	χ^2=9.14, P<0.01
Oecobius machadoi Males (Sintra)	Sintra	25	9	16	χ^2=1.96, NS	13	12	χ^2=0.04, NS
Oecobius amboseli	Algarve	20	10	10	χ^2=0.00, NS	12	8	χ^2=0.80, NS
Females (Kenya)	Sintra	23	15	8	χ^2=2.13, NS	10	13	χ^2=0.39, NS
Trachyzelotes bardiae	Algarve	26	13	13	χ^2=0.00, NS	13	13	χ^2=0.00, NS
Females (Sintra)	Sintra	24	18	6	χ^2=6.00, P<0.05	20	4	χ^2=10.67, P<0.01
Ctenolepisma sp.	Algarve	20	10	10	χ^2=0.00, NS	12	8	χ^2=0.80, NS
(Algarve & Sintra)	Sintra	21	13	8	χ^2=1.13, NS	12	9	χ^2=0.43, NS

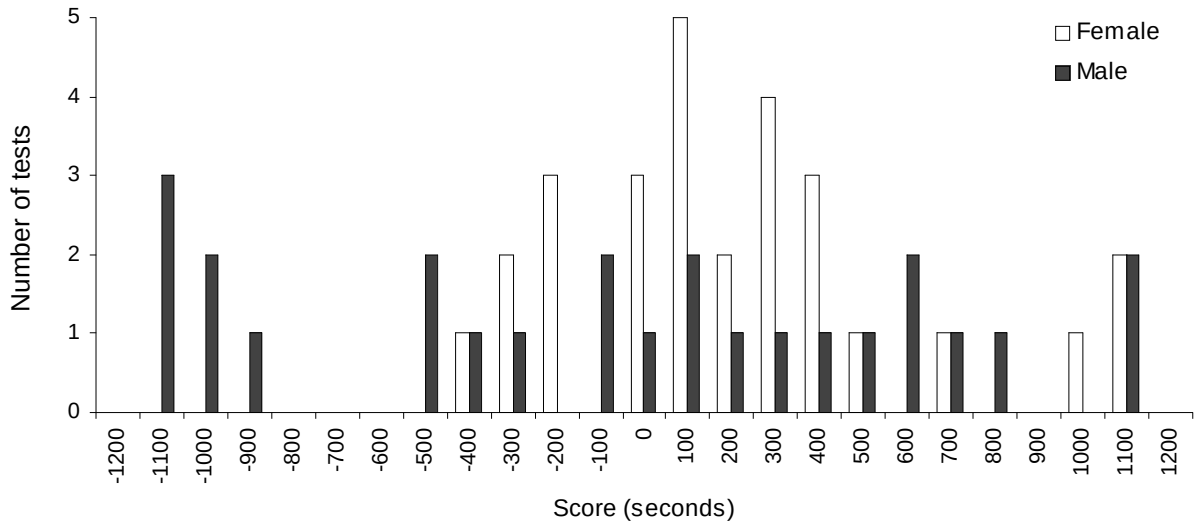

Figure 3. Difference scores from testing Sintra *Cyrba algerina* in olfactometer tests using female (white bars) and male *Oecobius machadoi* (black bars) as odour sources. Each individual provided a score (time spent on stimulus arm minus time spent on control arm). *C. algerina* spent significantly more time in the stimulus arm when *O. machadoi* females were used as an odour source (Wilcoxon signed-rank test, $P<0.01$, $N=28$) but not when the odour came from *O. machadoi* males (Wilcoxon signed-rank test, $P=0.84$, NS, $N=25$).

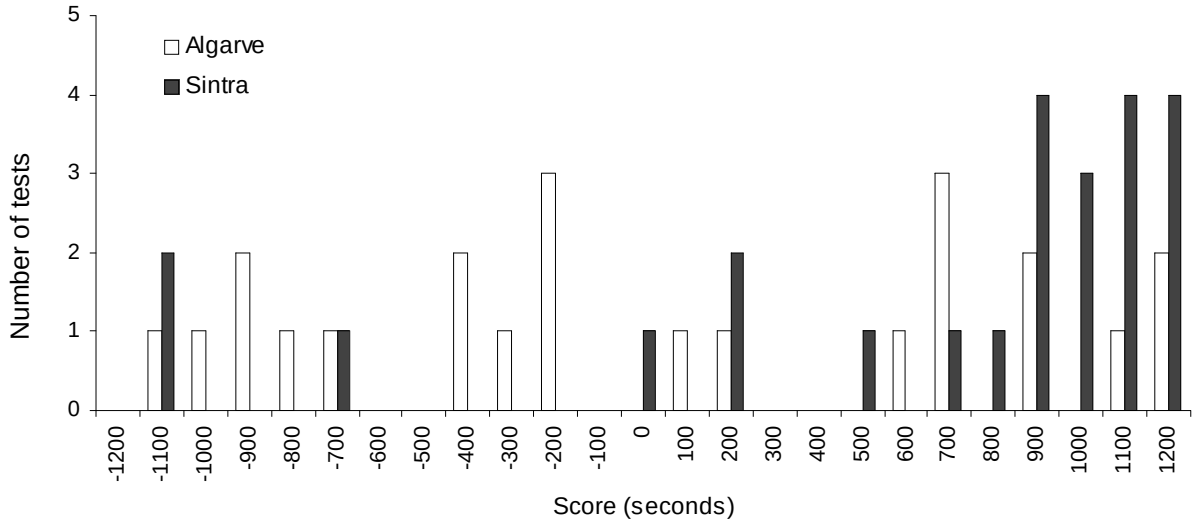

Figure 4. Difference scores from testing *Cyrba algerina* in olfactometer tests using *Trachyzelotes bardiae* as an odour source. Each individual provided a score (time spent on stimulus arm minus time spent on control arm). Sintra (black bars), but not the Algarve (white bars) *C. algerina*, spent significantly more time on the stimulus arm than on the control arm (Wilcoxon signed-rank tests; Sintra $P<0.01$, N=24; Algarve, $P=0.43$, NS, N=26).

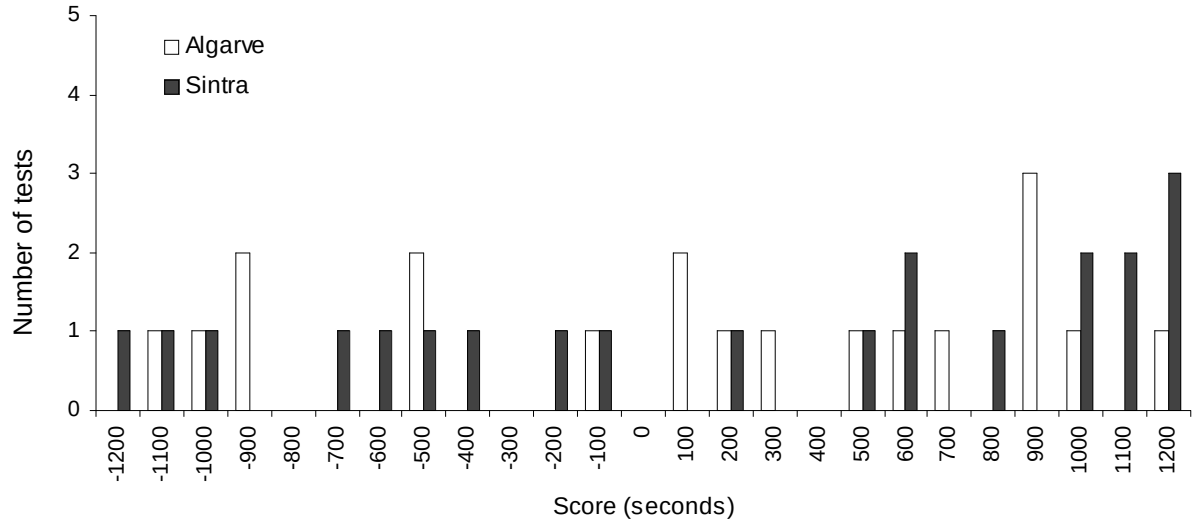

Figure 5. Difference scores from testing *Cyrba algerina* in olfactometer tests using bristletails (*Ctenolepisma* sp.) as an odour source. Each individual provided a score (time spent on stimulus arm minus time spent on control arm). Algarve (white bars) and Sintra (black bars) *C. algerina* did not spend significantly more time in either of the choice arms (Wilcoxon signed-rank tests: Algarve, P=0.72, NS, N=20; Sintra, P=0.22, NS, N=21).

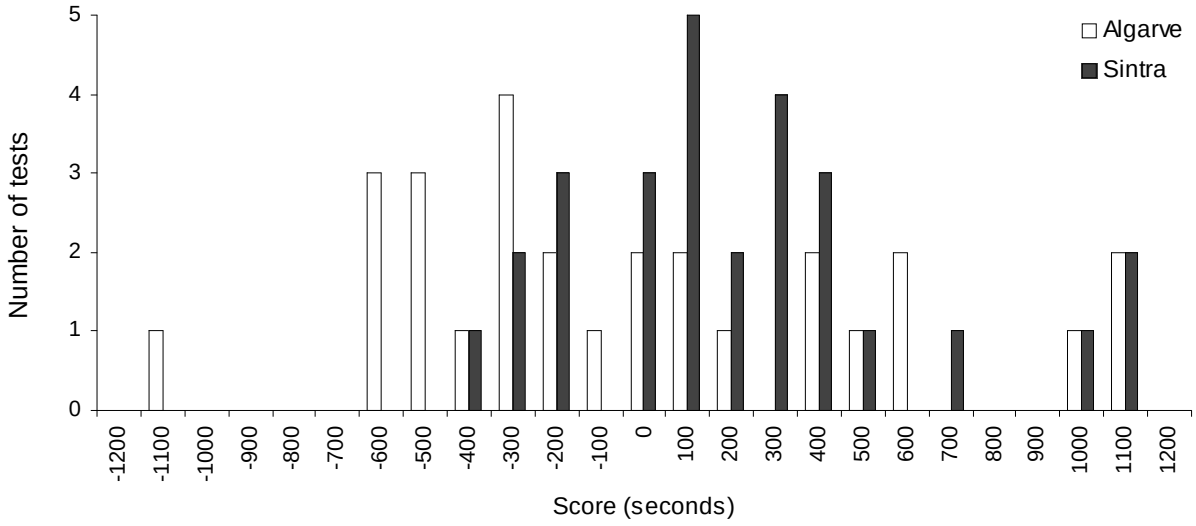

Figure 6. Difference scores from testing *Cyrba algerina* in olfactometer tests using *Oecobius machadoi* as an odour source. Each individual provided a score (time spent on stimulus arm minus time spent on control arm). Sintra (black bars), but not the Algarve (white bars) *C. algerina,* spent significantly more time on the stimulus arm than on the control arm (Wilcoxon signed-rank tests: Algarve, P=0.28, NS, N=28; Sintra, P<0.01, N=28).

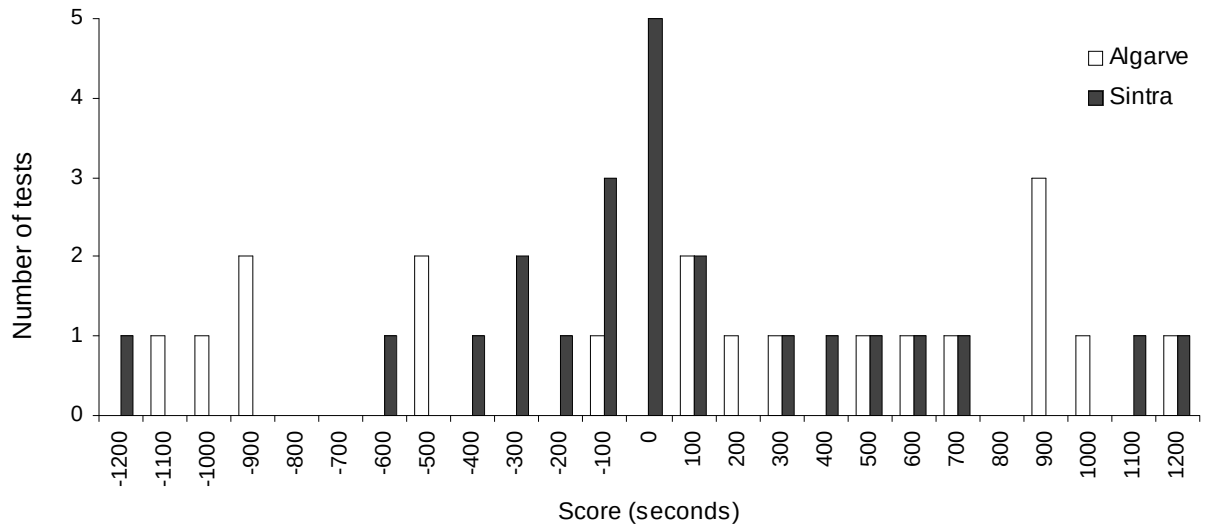

Figure 7. Difference scores from testing *Cyrba algerina* in olfactometer tests using *Oecobius amboseli* as an odour source. Each individual provided a score (time spent on stimulus arm minus time spent on control arm). Algarve (white bars) and Sintra (black bars) *C. algerina* did not spend significantly more time in either of the choice arms (Wilcoxon signed-rank tests: Algarve, P=0.72, NS, N=20; Sintra, P=0.75, NS, N=28).

Discussion

Kairomone use, in which a heterospecific receiver exploits the emission of a chemical compound by an emitter in its own benefit (Brown *et al* 1971, Schultz 2001), has been documented in a few spider species. For example, females of S*chizocosa ocreata* (Lycosidae) adjust the amount of time spent in a foraging patch based on the presence versus absence of substratum-borne chemical cues left by prey, such as that found in silk and faeces (Persons & Uetz 1996). The most interesting example is probably that of two wolf spiders (*Pardosa milvina* and *Hogna helluo*), where both prey and predator make use the other's chemical cues to detect each other's presence. The prey, *P. milvina,* shows reduced activity in the presence of silk and excreta cues from the predator, *H. helluo*, a behaviour known to greatly increase the probability of survival when in the presence of large *H. helluo* individuals (Persons & Rypstra 2001); the predator, *H. helluo,* can detect the presence of *P. milvina* on the basis of volatile and substrate-born chemical cues, and when in the presence of such cues decreases its activity, as part of its sit-and-wait foraging strategy (Persons & Rypstra 2000).

Examples of kairomone use during predatory encounters can also be found among salticids. *Habrocestum pulex*, an ant-eating salticid, has been shown to detect the presence of ant-derived chemical cues in the soil. Besides choosing to remain on soil containing chemical cues from ants, the presence of ant kairomones seems to stimulate *H. pulex* to adopt appropriate posture and behaviour for capturing ants. Kairomones from prey not only bring *H. pulex* into proximity with ants but also seem to prepare it for the encounter before an actual ant is seen. Kairomones also appear to influence *H. pulex*'s attention to optical cues from ants; when ant-derived cues are present, *H. pulex* locates ants faster than when they are absent (Clark *et al* 2000b).

Another advantage of kairomone use during predatory encounters is illustrated by the Queensland population of *Portia fimbriata*, a spider-eating jumping spider from Australia. Besides frequently preying on web-building spiders, the Queensland *P. fimbriata* also prefers other salticids to other spiders as prey. To capture them, Queensland *P. fimbriata* adopts a specialised predatory tactic known as cryptic stalking, not known to be used by any other population of *P. fimbriata* to capture this or any other type of prey. Queensland *P. fimbriata* appears to prey especially often on *Jacksonoides queenslandicus*, an especially abundant salticid in the Queensland habitat. Being the first to detect the other seems to be especially advantageous in this predator-prey system, as *J. queenslandicus* often flees or attacks approaching allopatric Portia that do not adopt cryptic stalking (Jackson & Hallas 1986).

Besides allowing *P. fimbriata* to detect the presence of an unseen *J. queenslandicus* in the surroundings, and to heighten its attention to optical cues from prey, the presence of kairomones also increases *P. fimbriata*'s inclination to adopt cryptic stalking (Jackson *et al* 2002), a behaviour which seems to be critically important in enabling *P. fimbriata* to be efficient at capturing *J. queenslandicus* as well as other salticids (Jackson & Hallas 1986).

The use of kairomones during predatory encounters seems to be highly advantageous. Similarly to *P. fimbriata* and *H. pulex*, reliance on chemical cues from common sympatric prey species may be highly advantageous to *C. algerina.* Besides allowing *C. algerina* to locate unseen prey in the surroundings, detection of kairomones from prey give the predator the element of surprise, allowing it to take measures to avoid being detected, to prepare itself for the encounter by adopting appropriate behaviour to capture *O. machadoi* and *T. bardiae* (Chapter 4), and even to become more attentive to particular cues from prey.

An additional advantage may apply to *C. algerina*. *C. algerina* lives in a very particular microhabitat, the undersides of stones. Low ambient light level is probably an important characteristic of this microhabitat. Although it is known whether predatory encounters are limited to this microhabitat, the fact that *C. algerina* was only rarely found in the open, and that its prey were also usually found on the undersides of stones, suggest that this was probably the case (Chapter 2). Therefore, compared to most salticids, *C. algerina* may not be able to rely so strongly on optical cues from prey during predatory encounters. Reliance on odour cues from common prey may be an especially important complement to optical cues in the detection and identification of prey under dim light conditions.

Although the Algarve and Sintra *C. algerina* feed on bristletails, and although bristletails are commonly found on both sites (Chapter 2), *C. algerina* individuals from either from either population did not approach the odour of this insect species. Sensitivity to the odour of bristletails seems, at least at first sight, advantageous; even though bristletails are usually larger than *C. algerina*, laboratory observations (Chapter 4) suggest that they can be considered "safe prey", in the sense that they never killed or injured *C. algerina* during predatory encounters. Their size could even be considered an advantage; preying on a bristletail would provide *C. algerina* with an especially big meal (i.e., *C. algerina* showed a greatly distended abdomen after feeding on bristletails) without the risk of getting preyed upon. Whether *C. algerina* is simply ignoring this species odour or whether it is unable to detect the prey's odour is unknown. However, another factor should be taken into consideration; being an araneophagic salticid, *C. algerina* may resemble *P. fimbriata* (Li & Jackson 1997) by being metabolically specialised at feeding on spiders, and it might need to include a great number of spiders in its diet to ensure a

proper development. If such is the case, *C. algerina*'s sensitivity to the odour of prey may be biased towards its most common and preferred prey group, spiders (Jackson & Li 1998, Guseinov *et al* 2004, Chapter 2), even if they provide smaller and more dangerous meals.

The fact that Sintra *C. algerina* was only attracted to the odour of one of the oecobiids used (*O. machadoi*) suggests that the odour cues *C. algerina* is using to detect this oecobiid species are species-specific. Additionally, *C. algerina* was only attracted to the odour cues of female *O. machadoi*, suggesting that *C. algerina* might be using this species' female pheromones as odour cues. Whether this is the case with *T. bardiae*, it is not known since only female individuals were used as odour sources.

The results obtained in this Chapter indicate that *C. algerina*'s attraction to the odour of particular prey species varies geographically, even over the short distances separating the Sintra and Algarve populations (c. 240 Km). Although only Sintra *C. algerina* approached the odour cues of sympatric spider species, this does not necessarily imply that only the Sintra individuals are influenced by chemical cues from prey. Because only a few prey species from the Algarve were used as odour sources, key prey species may have been overlooked.

Geographic variation in sensitivity to chemical cues from particular prey species has been shown to occur between the Queensland and the Northern Territory *P. fimbriata* populations (Jackson *et al* 2002). Because only second and third generation individuals reared in the laboratory were used, maternal effects and previous experience with prey were excluded as possible explanations for the observed variation between the populations of *P. fimbriata*, and the findings have been convincingly attributed to genetic divergence between the populations. Although the observed variation in the behaviour of *C. algerina*'s populations may also be innate (each population representing a behavioural ecotype adaptively fine tuned to respond to the odour cues of local abundant prey species), this cannot be concluded from the present results. In *C. algerina*'s case, because the individual's prior experience with prey is not known (the individuals used were collected from the field, not reared in the laboratory), an additional hypothesis should be considered; the variation in sensitivity to the odour of *O. machadoi* and *T. bardiae* could be a consequence of phenotypic plasticity, in which a single genotype fosters two or more phenotypes as a response to different environmental conditions. If such is the case, then each individual *C. algerina* from each population would be able to adapt its behaviour according to the prey available at each location based on its experience. Whether *C. algerina*'s predisposition to approach the odour from a particular prey is dependent on previous experience with prey or whether it is innate will be examined in a later Chapter.

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CHAPTER 6

Odour-based prey preference by *Cyrba algerina*

Abstract

Previous work has showed that *C. algerina* can detect between two sympatric spider species, *Oecobius machadoi* and *Trachyzelotes bardiae*, using odour cues alone. Using a Y-shaped olfactometer, experiments were carried out to investigate the ability of the Sintra *Cyrba algerina* to discriminate between these two sympatric spider species. The experimental findings suggest that the Sintra *C. algerina* has a preference for *T. bardei* as prey.

Introduction

Although the diet of an animal may suggest hypotheses about the animal's prey preferences, preference cannot simply be inferred directly from the animal's diet. Preference implies the ability to distinguish between different types of prey and choose to take one rather than the other (Morse 1980). What an animal actually eats, its diet, might be influenced by factors, such as prey availability and defences, and does not necessarily reflect the animals' preferences.

As for most spartaeines that have been studied, *Cyrba algerina* has a general preference for spiders over insects as prey (Li *et al* 1997, Jackson & Li 1998, Jackson 2000). More recent work has shown that, besides this general preference for spiders as prey, *C. algerina* from the Baku population has a specific preference for a particular spider, *Oecobius maculatus*, over other spiders (Guseinov *et al* 2004).

In the previous Chapter, I showed that Sintra *C. algerina* is attracted to the odour of two sympatric spider species, *Trachyzelotes bardiae* and *O. machadoi*. In this Chapter I investigate something more specific. My objective is to ascertain whether Sintra *C. algerina* can discriminate between the odour of these two spider species and whether it has an odour-based preference for one of these prey species over the other.

Methods

C. algerina individuals were tested in a Y-shaped olfactometer to assess their preference between two prey species on the basis of odour cues alone. Methods were as in Chapter 5 except that, instead of having a control (blank, no odour) and a stimulus chamber, there were two stimulus chambers, each containing one of the two prey species used as odour sources (i.e., the

two prey species were tested against each other). Two spider species were used as odour sources, *O. machadoi* and *T. bardiae*, assigned to the left or right testing chambers at random. The two choice arms will be referred to as the *O. machadoi* arm and the *T. bardiae* arm.

Data analysis

Chi-square tests for goodness of fit were used to analyse the spider's first and final choice (null hypothesis: both choice arms are chosen equally often). Scores were analysed using Wilcoxon signed-rank tests (null hypothesis: time spent in each choice arm equal) (Sokal & Rohlf 1995).

Results

Of the 26 *C. algerina* individuals tested, 20 (76%) chose the odour of *T. bardiae* as its first choice and 25 (96%) chose it as its final choice. *C. algerina* chose the odour of *T. bardiae* significantly more often than the odour of *O. machadoi* as its first (χ^2=7.54, P<0.01, N=26) as well as its final choice (χ^2=22.15, P<0.001, N=26)

Spiders also spent significantly more time in the *T. bardiae* arm than in the *O. machadoi* arm (Wilcoxon signed-rank test, P<0.001, N=26) (Fig. 1).

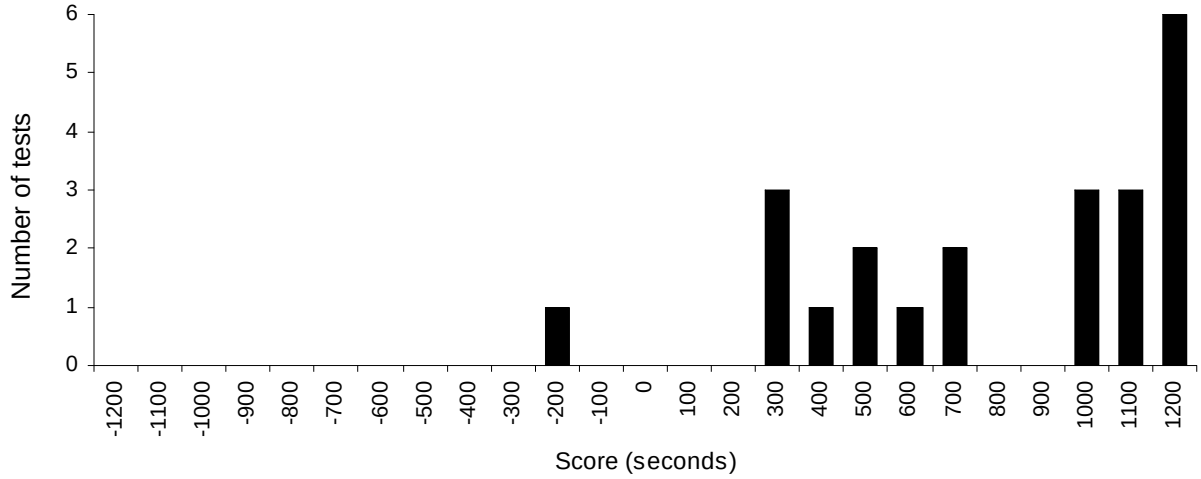

Figure 1. Difference scores from testing *Cyrba algerina's preference* in olfactometer tests using *Trachyzelotes bardiae* and *Oecobius machadoi* as odour sources. Each individual *Cyrba algerina* tested provided a score (time spent on the *T. bardiae* arm minus time spent on the *O. machadoi* arm). *C. algerina* spent significantly more time in the arm than in arm containing the odour of *T. bardiae* (N=26).

Discussion

Prey preference implies that the predator can detect and discriminate between different prey types, and actively chooses to take one rather than the other. Therefore, it would not be relevant to discuss "preference" when one of the hypothetical prey is not detected by the predator. This is one reason why the previous work was essential as a basis for the present Chapter, as it confirmed that the Sintra *C. algerina* can, in fact, detect the odour of both of the prey species used in the experiment reported on here.

The findings from this Chapter imply that the Sintra *C. algerina* makes odour-based discriminations between the two prey used, *Oecobius machadoi* and *Trachyzelotes bardiae*, and expresses a strong preference for *T. bardiae*. In *C. algerina*'s case, diet seems to reflect this species preferences, at least when it comes to spider prey; of all the instances Sintra *C. algerina* was found feeding in the field, *T. bardiae*, accounted for 32% of the total prey records, and 70% of the identifiable spiders captured by *C. algerina* (see Chapter 2).

The reasons behind *C. algerina*'s choosing *T. bardiae* in preference to *O. machadoi* are not known, but the literature on other predators suggests several hypotheses concerning factors that might influence *C. algerina*'s preference. These include: 1) prey size (Slootweg 1987, Török 1993); 2) the ratio between energy intake and foraging time (Ostfeld 1982, Brodmann & Reyer 1999); 3) specific nutrient requirements (Reichman 1977, Li & Jackson 1997); 4) the level of danger associated to the prey (Rissing 1984, Li & Jackson 1996); and 5) combinations of the various individual factors (see: Collins & McGrew 1985).

The ability to detect and discriminate between two prey species on the basis of their olfactory cues alone is also known to occur in other jumping spider species (Jackson *et al* 2005). Besides allowing the predator to prepare itself for the encounter, and potentially increasing the predator's chances to capture the prey (Clark *et al* 2000a), chemical cues from prey (kairomones) have been shown to be effective at making the predator more attentive to the prey's optical cues (Clark *et al* 2000b, Jackson *et al* 2002).

In *C. algerina*'s case reliance on kairomones from prey may be especially advantageous. *C. algerina* lives in a very particular microhabitat, the undersides of loose or partially buried stones, and field work (see Chapter 2) suggests that *C. algerina*'s predatory activity is largely restricted to the undersides of stones (i.e., *C. algerina* has never been found out in the open). Given the low light levels imposed by its microhabitat, the use *C. algerina* can make of vision is probably diminished, if not restricted to particular circumstances, compared to most typical salticids (i.e., compared to salticids that actively stalk prey in the open). Sensitivity to chemical

cues may therefore be an especially important complement to vision-based cues in the location and identification of prey under dim light levels.

As an araneophagic spider, *C. algerina* faces another challenge, as the roles of predator and prey can suddenly be reversed, and the hunter can easily become the hunted. However, in spite of being a spider, *O. machadoi* should not pose a great risk to *C. algerina*. Oecobiids are small spiders that spend most of their time under their star-shaped web. When prey (most often ants) is detected, the oecobiid rapidly rushes out of its web, and circles around the prey walking sideways while laying down silk over the prey, pinning it to the substrate. After the prey is secured (attached to the ground) the oecobiid bites the prey, and a few minutes later, after the prey becomes paralysed, takes it under its web to feed (Glatz 1967). The oecobiid's small size relative to *C. algerina*, combined with its particular predatory tactic, suggest that oecobiids are unlikely to cause an injury to *C. algerina*. During my observations when staging predatory encounters in the laboratory oecobiids never attempted to capture *C. algerina*, nor did *C. algerina* suffer any injury with either of the oecobiid species used.

In contrast, *T. bardiae* seems to be a more dangerous prey; adults can be similar in size or even bigger than *C. algerina*, and, unlike oecobiids, there were a few occasions in the laboratory when *T. bardiae* attacked, and even killed, *C. algerina* (see Chapter 4). Hence, it seems highly advantageous that *C. algerina*, besides being able to detect the presence of unseen prey in the environment, can discriminate between these prey species on the basis of odour cues alone. Having access to this information may allow *C. algerina* to plan ahead, and adopt an appropriate predatory tactic according to the prey species detected.

Although the present findings suggest that *C. algerina* has a preference for *T. bardiae* over *O. machadoi*, another possibility should, nevertheless, be taken into consideration. When investigating salticid sensitivity to a given odour, salticids are presented with a single odour. If the spider enters the choice arm containing the odour and remains in that arm for more than 30 s (Jackson *et al* 2002), (or in *C. algerina*'s case, the arm in which the spider spent most of its time during a 20-min period (see Chapter 5)), that is taken as evidence that the animal can detect the presence of that odour and is attracted to it. When testing for preference, the same rationale is applied, the main difference being that the spider is given an opportunity to choose between two different odours, and the spider's choice is taken as evidence of the spider's preference for one odour over the other. However, unless the exposure to the odour evokes an identifiable behavioural response in the animal being tested (such as the cryptic stalking posture adopted by Queensland *Portia fimbriata* when in the presence of odour from *Jacksonoides queesnlandicus*, see Jackson *et al* 2002), the validity of the preference test may be questioned, as it is not possible

for the observer to know whether the animal has in fact detected the presence of both odours. The possibility exists that one odour may be masking the other, in which case the spider would only be able to detect the masking but not the masked odour. Given that *C. algerina* behaved similarly to when tested simply with the odour of *T. bardiae* (see Chapter 5), the possibility that *T. bardiae*'s odour cues are masking the odour cues released by *O. machadoi* cannot be excluded. If this is the case, then *C. algerina*'s ability to detect the presence of *O. machadoi* in the olfactometer might be compromised, the results reported here simply reflecting the response to a masking odour and not the animal's actual preference. Additional work is necessary to test this hypoyhesis.

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CHAPTER 7

The effect of previous exposure to prey on *Cyrba algerina*'s prey preferences

Abstract

Previous results show that the Sintra, but not the Algarve population, of *Cyrba algerina* is attracted to the odour of a particular spider species, *Oecobius machadoi*. In this Chapter I examined whether this response to the odour of prey is influenced by previous experience with prey or, on the contrary, it is strictly innate (i.e., whether no prior experience of the odour is required before the response is expressed). The prey preferences of Sintra and Algarve populations of *C. algerina* were tested with sympatric and allopatric spider species in vision- and odour-based choice tests after a seven-day feeding period on one of three species: *Oecobius machadoi*, *O. amboseli* and *Nephylengys* sp. Results demonstrated that, after the feeding period *C. algerina* individuals from Sintra and the Algarve were visually and olfactorily attracted to sympatric and to allopatric *Oecobius* species, but individuals from neither of the populations were attracted either visually or olfactorily to *Nephylengys* sp. Exposure exclusively to the odour of allopatric *O. amboseli* (i.e., when *C. algerina* could not see the oecobiid but could smell it during training) elicited vision-based preference for this species in both Sintra and Algarve *C. algerina*, but there was no evidence of induced odour-based preference. These findings suggest that *C. algerina*'s sensitivity to prey, both visually and olfactorily, might be under the control of a developmental switch mechanism. Results indicate that previous experience with prey is necessary for *C. algerina* to manifest a preference for either of the oecobiid species used, and suggest that *C. algerina*'s populations might have an innate bias towards oecobiids as prey (i.e., a switch mechanism specific to oecobiids as prey).

Introduction

Individuals from populations subject to different conditions often exhibit interpopulation variation in behaviour, individuals from the different populations adopting different strategies more appropriate to the local circumstances. Although geographic variation in behaviour is often a consequence of underlying genetic differentiation (i.e., genetically-based variation in behaviour - behavioural ecotypes), it is also important to consider the possibility of geographic variation in

behaviour being primarily the result of phenotypic plasticity, a single genotype producing two or more phenotypes as a response to different environmental conditions (i.e., environmentally induced variation), or even the result of gene-by-environment interaction (i.e., geographic variation in phenotypic plasticity) (Stearns 1989, Carroll & Corneli 1999, Foster & Endler 1999, Thompson 1999).

In this Chapter, I investigate the underlying determinants of *Cyrba algerina*'s preference for oecobiids. *Oecobius machadoi* is a common spider species in the Sintra habitat of *Cyrba algerina*, but is apparently absent in the Algarve (Chapter 2). Results from a previous Chapter (Chapter 5) showed that only the Sintra population of *C. algerina* was attracted to the odour of *O. machadoi;* Algarve individuals did not approach this species' odour, suggesting that *C. algerina* is only attracted to the odour of sympatric oecobiid species.

The hypothesis I consider here is that *C. algerina*'s preference for a prey species is dependent on previous experience ("conditioning") with that particular prey. The prey preferences of *C. algerina*'s Sintra and Algarve individuals were tested on vision- and odour-based choice tests, after a seven-day feeding period ("direct conditioning") on one of the three following prey: *O. machadoi* (sympatric with the Sintra, but allopatric with the Algarve *C. algerina*), *O. amboseli* (allopatric with both the Sintra and the Algarve *C. algerina*) and a non-oecobiid prey spider, *Nephylengys* sp. (allopatric with both the Sintra and the Algarve *C. algerina*). Additionally, I considered the influence of oecobiid odour in the absence of experience preying on oecobiids ("odour conditioning") on *C. algerina*'s prey preferences by exposing *C. algerina* individuals exclusively to the odour of prey after a seven-day exposure period. The prey preferences of the Sintra and Algarve individuals of *C. algerina*'s were ascertained with vision- and odour-based choice testing. There were two types of conditioning: (1) direct conditioning, 7-day feeding period on one of three prey species (*O.* machadoi (sympatric with the Sintra, but allopatric with the Algarve *C. algerina*), *O. amboseli* (allopatric with both the Sintra and the Algarve *C. algerina*) and a non-oecobiid prey spider, *Nephylengys* sp. (allopatric with both the Sintra and the Algarve *C. algerina*)); (2) odour conditioning, 7-day period of exposure to odour of prey but not being able to see the prey.

Methods

Rearing

All *C. algerina* individuals used were reared in the laboratory from egg to maturity in environmentally enriched cages (as described in Chapter 3). However, for this experiment, spiders were housed individually to ensure that each individual had equal access to all types of

maintenance prey. Cages were inspected every day for webs built by spider prey and these webs were destroyed when found, as *C. algerina* tended to capture the prey more easily when the spider prey was away from its webs. Each *C. algerina* was provided with 2-3 lake flies (Diptera, Chironomidae, *Nilodorum brevibucca*) and 2-3 juveniles *Nephilengys* sp. (Nephilidae) (referred to as the "standard diet"). Each prey was smaller than *C. algerina* in body length. Feeding was *ad libitum* (i.e., the stated types of prey were always available to *C. algerina* in its cage), as *C. algerina* tended to have an easier time capturing prey spiders that were away from webs.

The prey used was always smaller than *C. algerina* individuals in terms of body length. However, in the unusual event of a juvenile prey spider put in a cage with *C. algerina* surviving long enough to grow to *C. algerina*'s size, the prey spider was removed from *C. algerina*'s cage and replaced it with a smaller individual of the same prey-spider species.

Direct conditioning

Adult females of *C. algerina* from the Algarve and Sintra were randomly assigned to one of three groups. Group 1 was fed the standard diet (control), group 2 was fed the standard diet plus *O. machadoi* (Oecobiidae) and group 3 was fed the standard diet plus *O. amboseli*. Each group was fed the specified prey for 7 days.

Cages were checked daily and topped up with the required prey, thereby ensuring that *C. algerina* always had access to each specified type of prey. A total of 4-6 individual prey items was always present in the cage, with the numbers being 1-2 for each of the three prey types (e.g., *Oecobius* spp. plus *Nephilengys* sp. and lake flies). Except for *O. machadoi*, all prey used were from Mbita Point, Kenya, and were collected in the field (Mbita Point) as needed. *O. machadoi* came from laboratory cultures. Only adult females of *O. machadoi* and *O. amboseli* were used.

After the 7-day exposure period, *C. algerina* individuals were submitted to a 7-day fast to ensure that the spiders were motivated to feed during testing, and tested for vision- and odour-based choice.

Odour conditioning

The apparatus for exposing *C. algerina* to the odour of prey was a modification of the standard rearing cage. Three additional holes (8 mm in diameter) were made in each cage, one on top of the cage and two in the middle of the sides of the cage, about halfway between the bottom and the top, on opposite sides of the cage. A 15 mm long glass tube was inserted in each of the holes. Each tube was attached through a rubber stopper to a second tube (75 mm long and 12 mm wide), the "odour chamber". The two openings of the glass tube were covered with fine

mosquito netting to prevent physical contact between *C. algerina* and the prey used as an odour source. Odour entered *C. algerina*'s cage by diffusion via the glass tubes. Odour chambers and stoppers were painted black to prevent *C. algerina* from seeing the odour-source spiders.

Adult females of *C. algerina* from the Algarve and Sintra populations were randomly assigned to one of two groups. Group 1 was exposed to the odour of *O. amboseli* and group 2 was exposed to the odour of *Nephilengys* sp. Each odour chamber contained two adult females of *O. amboseli* (Group 1) or two juveniles of *Nephilengys* sp. (Group 2). The spiders used as odour sources were released in the field and replaced by freshly collected individuals every 2 days. Both *C. algerina* groups were fed the standard diet while being exposed to the odour of *O. amboseli* and *Nephilengys* sp.

After a 7-day exposure period, the odours chambers were emptied and *C. algerina* individuals were submitted to a 7-day fast to ensure that the spiders were motivated to feed during testing. *C. algerina* individuals were then tested for vision- and odour-based choice using *O. amboseli* and *Pardosa messingerae* as prey.

Testing for vision-based prey choice

Lures were made by first immobilising prey individuals with carbon dioxide and then placing prey in ethanol for 60 min. Prey was then mounted in lifelike posture on the centre of a disc-shaped piece of cork (diameter of the cork disc 1.25 times the body length of the prey) and sprayed with an aerosol plastic adhesive for preservation (for details see Li & Jackson 1996). Between tests, the apparatus was washed with 80% ethanol followed by distilled water and then dried. Each individual *C. algerina* was tested only once.

The testing apparatus was a shallow, rectangular box (140 mm long X 115 mm wide, 20 mm deep) made of transparent Perspex and covered with a transparent glass lid (Fig. 1). There were two holes (diameter 8 mm) in the base of the box (left and right lure hole). The left hole was located 10 mm from the longer left side of the box and right hole was located 10 mm from the longer right side of the box (measured from the nearest side of the hole). Both holes were 10 mm from one of the two narrower sides (same side for both holes) of the box. Lures on cork disks sat on top of left and right holes (i.e., the diameter of cork disc was wider than diameter of hole), facing directly away from the closer wall. A stiff piece of wire was attached to the bottom of each cork disc. The two wire pieces were the prongs of a 2-prong metal fork, with the handle being a camera cable-release cord.

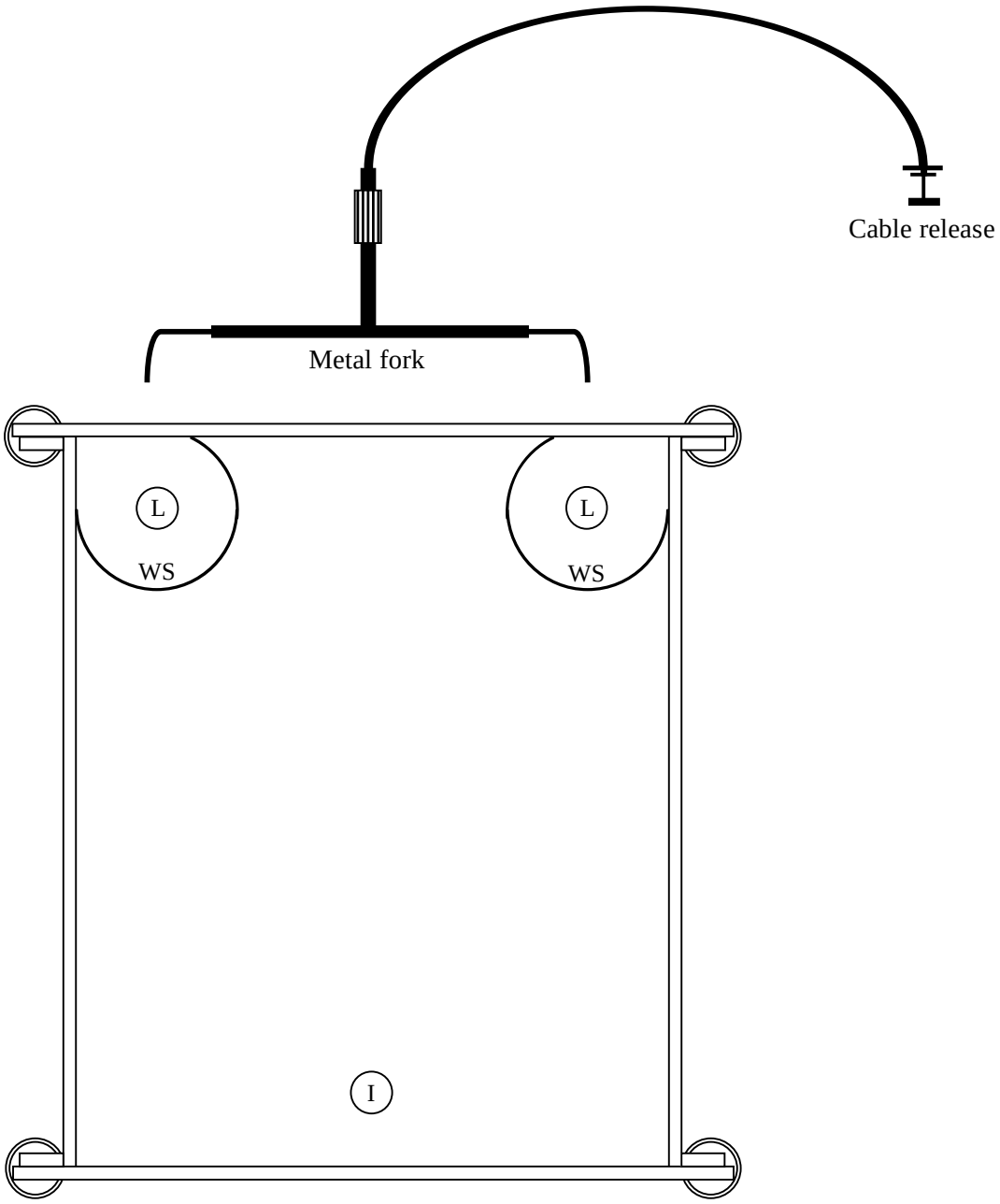

Figure 1. Apparatus for vision-based choice tests (viewed from above). *Cyrba algerina* introduced to apparatus through introduction hole (I) and allowed to approach lures (L). First wire semicircle (WS) *C. algerina* entered was recorded as choice. Lures moved by pressing cable release (see text for details).

The box apparatus sat on top of a Perspex stand (height 150 mm), with the end of the cable release being accessible from beneath the stand. By pressing on the cable release, the two prongs on the metal fork lifted the two lures simultaneously 5 mm above the floor of the Perspex box. Each of the two lures was made from a different spider species (which species was on the left or the right hole determined at random for each trial). During each trial, the cable release was pressed once every 30 s and then immediately released, causing the two lures to move up once and down once simultaneously.

Test spiders were introduced in the apparatus through the "introduction hole" (diameter 8 mm) in the base of the box. The introduction hole was equidistant from the two longer sides of the box, its inner edge being 10 mm from the narrow side of the box at the opposite end from the lures. Before testing began, an individual of *C. algerina* (the "test spider") was taken into a plastic tube (20 mm long, diameter 8 mm and its two ends plugged with corks. After a 15-min acclimatisation period, one of the corks was removed and the end of the tube was fit snugly into the introduction hole. *C. algerina* usually walked spontaneously out of the tube and into the box within 10 min after the tube was connected to the apparatus. However, if the test individual was still in the tube after 10 min, the other cork was removed and a soft brush was slowly inserted to entice *C. algerina* out into the box. The routine of moving the lures each 30 s began as soon as *C. algerina* was inside the box.

Two semicircles (radius 18 mm) of wire were placed on the bottom of the box, one encircling the right lure hole and the other enclosing the left lure hole (centre of the hole and centre of hole 18 mm from wire loop). When a test spider entered one of the two circles, this was recorded as its choice. The test spider was allowed 60 min in which to make a choice with a condition that, if it were oriented toward a lure, but not inside one of the semicircles when the 60-min period expired, then testing continued until it made its choice or it turned away for 30 s.

<u>Testing for odour-based prey choice</u>

C. algerina's response to specific odours was assessed by using a Y-shaped olfactometer as described in Chapter 5, except that during each trial two odour sources, instead of one, were used (i.e., each stimulus chamber contained an odour source). Additionally, each choice arm was covered with a tight sleeve made from black paper. Having the sleeve meant that the test spider began the test in the light (holding chamber) and then chose a choice arm by entering one of two dark chambers. The olfactometer was also placed inside a brown cardboard box, from which the front end had been cut away, to give the experimenter easy access. Small holes at the distal end

of the box allowed the plastic tubing connected to the pump (kept outside the box) to enter the box. With the light source being outside the box, the level of ambient light was much reduced.

The rationale for using the box and the sleeves (these two being referred to jointly as "shielding") was related with *C. algerina*'s tendency to move away from light. During the olfactometer testing in earlier chapters (Chapter 5 & 6), which was done under the normal levels of light in the laboratory without shielding, *C. algerina* sometimes remained quiescent for highly variable periods in the holding chamber and then suddenly ran into one or the other of the choice arms. This may explain why 'first choice' was not informative in the earlier work and why it was necessary to record how much time the test spider spent in each stimulus arm. However, when under shielding, *C. algerina* behaved more calmly and usually remained in the arm it first entered. Test spiders had 30 min to make a choice (definition: entered a choice arm and remained there for 30 s). Earlier olfactometer studies have shown that this 30-s rule is reliable for other salticid species, and our preliminary testing confirmed that it was reliable for *C. algerina*, but only after making the above modifications of the apparatus. These modifications allowed testing a much greater number of individuals than in previous chapters in a short amount of time without negatively affecting the results.

Persistence of the effect of exposure to prey

The persistence of *C. algerina*'s preference was assessed by repeating the vision- and odour-based prey-choice tests. This was done by taking a random subset of the individuals that had been previously used in direct conditioning on *O. amboseli* and testing them again 8 weeks after their preference was first tested. Individuals that had been direct conditioned on *O. machadoi* were not tested because of insufficient numbers of this prey.

A group of individuals from Sintra and another group from the Algarve were used for testing persistence of vision-based preference. Another two groups of individuals, each from one of the two populations, were used for testing persistence of odour-based preference (i.e., the individuals used for testing the persistence of vision-based preference had been used in vision-based testing before. The same applies to individuals used foe testing odour-based preference). Only individuals that had chosen *O. amboseli* over *Nephilengys sp.* were used.

Spiders were maintained on the standard diet for 8 weeks after they had first been tested for vision- based or odour-based preference. This was followed by a 7-day fast period. Spiders were then tested with *O. amboseli* and *Nephilengys* sp. for vision- and odour-based preference a second time (methods described above).

Data analysis

All data were analysed using chi-square tests for goodness of fit (null hypothesis: spiders chose either prey type as equally often as the other) (see Sokal & Rohlf 1995).

Results

Direct conditioning

When *C. algerina* individuals reared on the standard diet only (i.e., when the only spider prey to which they had access to was *Nephilengys* sp.) were given a choice between *O. amboseli* and *Nephilengys* sp., on the basis optical cues alone, individuals of *C. algerina* from neither population chose either prey significantly more often than the other (Table 1). However, when given a choice between *Nephilengys* sp. and either one of the *Oecobius* spp. to which it had been exposed, *C. algerina* from both populations always chose the *Oecobius* species to which they had been exposed significantly more often than they chose *Nephilengys* sp.

The results obtained from conditioning *C. algerina* individuals to *O. amboseli* and to *O. machadoi* were not significantly different; *C. algerina* from Algarve did not chose either one of the oecobiid species used significantly more often on the basis of optical cues alone (test of independence: χ^2=2.96, NS, N=60), nor did *C. algerina* individuals from the Sintra population (test of independence: χ^2=2.96, NS, N=60).

Results from odour-based choice were similar to those obtained from vision-based choice. Regardless of the *Oecobius* species used, *C. algerina* individuals from both populations chose the odour of *O. amboseli* or the odour of *O. machadoi*, significantly more often than the odour of *Nephilengys* sp. (Table 2). In contrast, individuals reared on the standard di*et al*one, did not approach the odour of either prey significantly more often than the other.

C. algerina individuals from both populations did not chose the odour of either of the oecobiid species to which they were conditioned in relation to the odour of *Nephilengys* sp. significantly more often than the other (tests of independence: Algarve *C. algerina*, χ^2=0.80, NS, N=60; Sintra *C. algerina*, χ^2=1.92, NS, N=60).

Table 1. Vision-based prey choice by *Cyrba algerina* after direct conditioning. During testing, *C. algerina* had simultaneous access to one lure made from an oecobiid (*Oecobius amboseli*, *O. machadoi*) and another lure made from *Nephilengys* sp.

Population of *C. algerina*	Prey used for direct conditioning	*Oecobius* species	Chose *Oecobius*	Chose *Nephilengys*	N	Chi-square tests of goodness of fit*
Algarve	*Nephilengys* sp.	*O. amboseli*	30	20	50	χ^2=2.00, NS
	O. amboseli	*O. amboseli*	25	5	30	χ^2=13.33, P<0.001
	O. machadoi	*O. machadoi*	29	1	30	χ^2=26.13, P<0.001
Sintra	*Nephilengys* sp.	*O. amboseli*	21	29	50	χ^2=1.28, NS
	O. amboseli	*O. amboseli*	29	1	30	χ^2=26.13, P<0.001
	O. machadoi	*O. machadoi*	25	5	30	χ^2=13.33, P<0.001

* Null hypothesis: *C. algerina* chose *Oecobius* spp. and *Nephilengys* sp. equally often

Table 2. Odour-based prey choice by *Cyrba algerina* after direct conditioning. During testing, *C. algerina* had simultaneous access to one lure made from an oecobiid (*Oecobius amboseli*, *O. machadoi*) and another lure made from *Nephilengys* sp.

Population of *C. algerina*	Prey used for direct conditioning	*Oecobius* species	Chose *Oecobius*	Chose *Nephilengys*	N	Chi-square tests of goodness of fit*
Algarve	*Nephilengys* sp.	*O. amboseli*	23	27	50	χ^2=0.32, NS
	O. amboseli	*O. amboseli*	21	9	30	χ^2=4.80, P<0.05
	O. machadoi	*O. machadoi*	24	6	30	χ^2=10.80, P<0.01
Sintra	*Nephilengys* sp.	*O. amboseli*	27	23	50	χ^2=0.32, NS
	O. amboseli	*O. amboseli*	27	3	30	χ^2=19.20, P<0.001
	O. machadoi	*O. machadoi*	23	7	30	χ^2=8.53, P<0.01

*Null hypothesis: *C. algerina* chose *Oecobius* spp. and *Nephilengys* sp. equally often

Persistence of prey choice behaviour

C. algerina's vision- and odour-based preferences persisted after the 8-week period without access to the prey (*O. amboseli*) used for conditioning . After the 8-week period without access to *O. amboseli*, *C. algerina* still maintained its vision- and odour-based preferences; *C. algerina* individuals from both populations chose *O. amboseli* on the basis of optical cues alone significantly more often than *Nephilengys* sp. (Table 3). The same was true when the prey choice was based solely on the odour cues from prey; *C. algerina* from both populations chose the odour of *O. amboseli* significantly more often than the odour of *Nephilengys* sp.

C. algerina's preference was not significantly affected by the type of cues used in the choice of prey; the choice of Algarve and Sintra individuals when tested with optical cues was not significantly different from when tested with odour cues (tests of independence: Algarve, χ^2=1.15, NS, N=30; Sintra, χ^2=1.56, NS, N=40).

C. algerina's odour-based choices immediately after the 7-day conditioning period were not significantly different from the choices after 8 weeks (tests of independence: Algarve, χ^2=0.51, NS, N=45; Sintra χ^2=2.01, NS, N=50). The same was true for vision-based choices (tests of independence: Algarve, χ^2=0.87, NS, N=45; Sintra, χ^2=0.95, NS, N=50).

Odour conditioning

When *C. algerina* individuals conditioned on the odour of *Nephilengys* sp. (control) were given a choice between *O. amboseli* and *P. messingerae*, *C. algerina* from both populations did not chose either prey significantly more often than the other based on the preys' visual or olfactory cues (Table 4 & 5).

Similarly, when *C. algerina* individuals conditioned to the odour of *O. amboseli* were given a choice between the odour of *O. amboseli* and *P. messingerae*, neither of the populations chose any prey significantly more often than the other (Table 5). However, when the choice was based on the prey's visual cues, *C. algerina* individuals chose *O. amboseli* significantly more often than *P. messingerae* (Table 4) after conditioning on oecobiid odour.

There was no evidence that conditioning on prey odour affected *C. algerina*'s odour-based preferences; for both populations, and regardless of the prey odour on which *C. algerina* had been conditioned, how often the two prey types in odour-based tests were chosen were not significantly different (test of independence: Algarve, χ^2=1.06, NS, N=98; Sintra, χ^2=1.46, NS, N=178).

However, when choice was based on visual cues, the prey odour on which *C. algerina* had been conditioned influenced the prey chosen (tests of independence: Algarve, χ^2=4.52, P<0.05, N=94; Sintra, χ^2=5.94, P<0.05, N=151).

Table 3. Persistence of vision- and odour-based prey-choice by *Cyrba algerina* 8 weeks after direct conditioning on *Oecobius amboseli*. During testing, *C. algerina* had simultaneous access to cues from *O. amboseli* and *Nephilengys* sp.

Type of choice test	Population of *C. algerina*	Chose *O. amboseli*	Chose *Nephilengys* sp.	N	Chi-square tests of goodness of fit*
Vision-based	Algarve	14	1	15	χ^2=11.27, P<0.001
prey choice	Sintra	18	2	20	χ^2=12.80, P<0.001
Odour-based	Algarve	12	3	15	χ^2=5.40, P=0.020
prey choice	Sintra	15	5	20	χ^2=5.00, P=0.025

* Null hypothesis: *C. algerina* chose *O. amboseli* and *Nephilengys* sp. equally often.

Table 4. Vision-based prey choice by *Cyrba algerina* individuals from the Sintra and Algarve populations after odour conditioning with *Nephilengys* sp. and *Oecobius amboseli*. During testing, *C. algerina* had simultaneous access to one lure made from *O. amboseli* and another made from *Pardosa messingerae*.

Population of *C. algerina*	Prey used for odour conditioning	Chose *O. amboseli*	Chose *P. messingerae*	N	Chi-square tests of goodness of fit*
Algarve	*Nephilengys* sp.	28	21	49	χ^2=1.00, NS
	O. amboseli	35	10	45	χ^2=13.89, P<0.001
Sintra	*Nephilengys* sp.	42	38	80	χ^2=0.20, NS
	O. amboseli	51	20	71	χ^2=13.54, P<0.001

* Null hypothesis: *C. algerina* chose *O. amboseli* and *P. messingerae* equally often.

Table 5. Odour-based prey choice by *Cyrba algerina* individuals from the Sintra and Algarve populations after odour conditioning with *Nephilengys* sp. and *Oecobius amboseli*. During testing, *C. algerina* had simultaneous access to odour cues from *O. amboseli* and from *Pardosa messingerae*.

Population of *C. algerina*	Prey used for odour conditioning	Chose *O. amboseli*	Chose *P. messingerae*	N	Chi-squared tests of goodness of fit*
Algarve	*Nephilengys* sp.	24	27	51	χ^2=0.18, NS
	O. amboseli	27	20	47	χ^2=1.04, NS
Sintra	*Nephilengys* sp.	43	47	90	χ^2=0.18, NS
	O. amboseli	50	38	88	χ^2=1.64, NS

* Null hypothesis: *C. algerina* chose *O. amboseli* and *P. messingerae* equally often.

Discussion

The findings here presented provide evidence that at least some level of previous experience with prey is necessary for *C. algerina* to manifest a preference for a given type of prey, both olfactorily and visually. However, the acquisition of preference for prey did not extend to all prey species *C. algerina* was conditioned; *C. algerina* did not manifest a preference for *Nephylengys* sp. in spite of being equally conditioned on this spider. The fact that *C. algerina* from Sintra and Algarve were both attracted to an allopatric oecobiid species (i.e., *O. amboseli* for Sintra *C. algerina* and *O. machadoi* and *O. amboseli* for the Algarve *C. algerina*) suggests that *C. algerina* might be biased to respond to olfactory and optical cues from this particular spider family.

A preference for *O. amboseli*, a Kenyan species not likely to be encountered in nature by any of *C. algerina* (i.e., the distributions of the two species do not overlap), over *Nephylengys* sp. is probably related with visual and olfactory similarities between *O. machadoi* and *O. amboseli*. At least to the human observer, *O. machadoi* and *O. amboseli* are very similar in appearance; the two species adopt similar leg postures, are similar in size and have only minor differences in coloration. Although nothing is known about the identity of the volatile compounds responsible for oecobiid odour, results from Chapter 5 indicate that *C. algerina* categorises the odour of *O. amboseli* and *O. machadoi* as different. It is often the case with animal pheromones that related species use the same chemical compounds to produce a chemical selective signal simply by using different blends of the same chemical compounds. This is especially true in species, which are separated by different patterns of activity or, as is the case of *O. machadoi* and *O. amboseli*, in space, as the need for especially high levels of specificity in the signal is much lower (Schulz 2001). If this trend holds true for the compound blends responsible for the odour of *Oecobius* species, then it is possible that *C. algerina* is capable responding to different *Oecobius* species by flexibly adjusting its sensory system through experience with locally available species.

It is interesting that *C. algerina* conditioned on *Nephylengys* sp. did not approach *Nephylengys* sp. significantly more often than *O. machadoi* or *O. amboseli*. Perhaps *C. algerina* is incapable of detecting the odour of *Nephylengys* sp. Alternatively, *C. algerina* might detect the odour of *Nephylengys* sp., all the while not being predisposed to being conditioned on this odour. A third alternative is that *C. algerina* is subject to having its behaviour modified by exposure to the odour of *Nephilengys* sp. but the testing methods adopted were not adequate for demonstrating this.

Food imprinting (Burghardt & Hess 1966, Apflebach 1986, Punzo 2002, Darmaillacq *et al* 2006a,b) and associative learning (Daly & Smith 2000, Persons & Rypstra 2000, Cunningham

et al 2004) are the two most common processes through which animals are known to acquire or alter food preferences. Although imprinting was initially considered a special form of learning and considered to be very different from associative learning, as more evidence became available, and the similarities between imprinting and other learning processes demonstrated (Hollis e*t al* 1991), this view went out of favour. Yet there are still two criteria that are strongly associated with imprinting. These are the existence of a sensitive period (i.e., a restricted period of the individuals' life during which the learning process takes place), and the subsequent stability of the behaviour in question, resultant from a particular experience during the sensitive period (Immelmann 1975).

Although food imprinting has been reported in the snapping turtle *Chelydra serpentina* (Burghardt & Hess 1966), and in the lynx spider *Oxyopes salticus* (Punzo 2002), it is questionable whether this term is actually applicable to these studie cases. Both studies show that experience (i.e., feeding) early in life with a particular prey type influence the animal's subsequent preference. However, neither of the studies clearly demonstrates the existence of a sensitive period in the acquisition of a preference given that the effect of exposure to prey at a later stage in the animals' development was not investigated. Therefore, it is not possible to be sure whether a sensitive period really exists in either of these species (see Darmaillacq *et al* 2006b) or, on the contrary, whether the acquisition of a preference for a specific food type would occur at any given stage of the animal's development if given the chance (see Grassman & Owens 1982). Because no further experiments considering the effect of exposure to food items at a later developmental stage were carried, it seems that in both studies the assumption that a sensitive period existed, luckily matching the exposure period adopted by the experimenters, was made.

Whether *C. algerina*'s acquisition of preferences is subject to a sensitive period is unknown, and was not investigated here. However, the experiments were done with conditioning being late in *C. algerina*'s life (immediately after maturity), suggesting that a sensitive period was not involved. Although the possibility of a sensitive period after maturation is not incompatible with imprinting, better-known examples of sensitive periods occur early in the animal's life and are short in duration (Immelmann 1975). Therefore, it seems unlikely that the acquisition of *C. algerina*'s food preferences is a result of food imprinting.

Were it not for the findings from the odour-conditioning experiment, it would be easy to propose that *C. algerina*'s odour- and vision-based preferences is a result of conventional associative learning (i.e., an ability to acquire conditioned responses by associating neutral with significant stimuli). However, the findings do not actually imply that *C. algerina* associated the

olfactory and visual cues of prey with a reward (i.e., ingestion of the actual prey). Instead, what was found was that odour-conditioned *C. algerina* later showed a vision-based preference for *O. amboseli*, a prey which *C. algerina* had never seen before.

Developmental switches are a mechanism for phenotypic plasticity (i.e., the capacity of a single genotype to produce a range of phenotypes in response to different environmental conditions (Bradshaw 1965)). After a period of neglect, the study of phenotypic plasticity is becoming increasingly common, numerous studies documenting the occurrence of phenotypic plasticity (Greene 1989, Sorci *et al* 1996, Relyea 2003, 2004, Aubret *et al* 2004, Pigliucci 1996, 2005, Nussey *et al* 2005, Postma & van Noordwijk 2005, Iraeta *et al* 2006, to name a few), and the mechanisms behind its evolution (Schlichting 1986, 1989, Stearns 1989, West-Eberhard 1989, Hazel *et al* 1990, Scheiner 1993, 1998, Via *et al* 1995).

Phenotypic variation may be expressed as a continuum, with the phenotype being a continuous function of an environmental signal. In these instances, the term "reaction norm" is often used. An especially thoroughly studied behavioural example is that of the great tits. These birds time their reproduction so as to synchronise it with the growth rates of caterpillars on which they feed their young (see Nussey *et al* 2005 for more details). Alternatively, phenotypic variation may be discrete, a single genotype producing two or more discrete phenotypes in response to different environmental signals (i.e., developmental switches) (Stearns 1989, Krebs & Davies 1991). A well-known example of discrete phenotypic variation is the environmental sex determination system that applies to more than 70 species of reptiles, egg incubation temperature determining the sex of these reptiles (see Ciofi & Swingland 1997 for a review). Rather than conventional associative learning, the mechanism involved in the formation of *C. algerina*'s preferences seems to be akin to a developmental switch that was "switched on" after contact with oecobiids.

The findings from this chapter suggest that *C. algerina*'s acquisition of preference for oecobiids is under the control of a developmental switch, analogous to the switch that determines the sex of certain reptiles. Encountering and preying on oecobiids appears to trigger this innate switch mechanism in both Algarve and Sintra individuals. Additionally, the switch mechanism appears to be specific to oecobiids. That is, *C. algerina* does not appear to be predisposed with switches to just any spider, as there was no evidence of a switch being triggered by conditioning with *Nephylengys* sp. Such specificity towards oecobiids is not surprising; although *C. algerina* was never found feeding on *O. machadoi* in the field, *O. machadoi* is one of the most common spider species in *C. algerina*'s habitat in Sintra (Chapter 2). The fact that Sintra *C. algerina* is clearly able detect this species' presence using olfactory cues (Chapter 5), and has a specific

predatory tactic to capture this prey (Chapter 4), suggest that *O. machadoi* an is especially important prey to Sintra *C. algerina*. In contrast, *Nephylengys* sp. is an African species, not known to occur in the Sintra or the Algarve habitats. Of course, more prey need to be used in conditioning experiments in order to determine how specifically tuned the switching mechanism might be. In particular, experiments should be done with other biologically relevant species such as *Trachyzelotes bardiae* (see Chapter 5 & 6), a common prey species of Sintra *C. algerina* (Chapter 2).

It is interesting that vision-based, but not odour-based, preference was detected (i.e., odour alone appears to be sufficient to trigger the switch for vision-based preference, but apparently not for odour-based preference) after conditioning on odour. Understanding why this was so will require additional research. One possibility is that *C. algerina* requires more direct experience (perhaps actually eating of the oecobiid) before odour-based preference is triggered, whereas vision-based preference is more easily induced. Alternatively, it might be that different testing methods would have detected odour-based preference after odour conditioning alone.

Earlier work (Chapter 5) showed that there is interpopulation variation in how Sintra and Algarve *C. algerina* respond to oecobiids, but the mechanism underlying this variation was not clear because only field-collected spiders were used. However, the findings in this Chapter imply that both populations are sensitive to conditioning with oecobiids (i.e., preference for oecobiids was triggered in both populations). This does not rule out the possibility that there is ecotypic variation in how predisposed different populations might be to having developmental switches triggered by particular oecobiid species, but these findings, nonetheless, illustrate that phenotypic plasticity is an important factor behind the interpopulation variation in prey-choice behaviour that occurs in *C. algerina*.

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CHAPTER 8

Optics and histology of the principal eye of *Cyrba algerina* - adaptations to dim light?

Abstract

The retinal ultrastructure of the principal eye of the primitive salticid spider *Cyrba algerina* is considered to be less organised than that of typical ("advanced") salticids and, consequently, it has been suggested that *C. algerina*'s retinal mosaic may represent an intermediate stage in the evolution towards the high spatially-acute retinal mosaics found in modern salticids. Although *C. algerina*'s twin rhabdomere arrangement might be detrimental in terms of spatial resolution, this same arrangement should be advantageous in terms of sensitivity to light. The dim light levels as well as a low diversity of prey found in *C. algerina*'s habitat might have favoured the retention of a retinal mosaic that emphasizes sensitivity at the expense of spatial acuity. By having a short focal length, reduced power of the diverging component, wider and contiguous adjacent rhabdomeres, *C. algerina*'s principal eyes seem to be able to minimise the visual constraints imposed by the low light levels of its microhabitat.

Introduction

Seeing well is as much a matter of resolution (i.e., the ability to resolve fine detail in space and time) as it is of sensitivity to light (i.e., the amount of light an eye is able to capture). An eye's spatial resolution depends on photoreceptor width and inter-receptor spacing of the retinal mosaic. When an image falls upon the retina and is sampled by photoreceptors, each receptor samples a specific part of the image. If the receptors are wide and far apart, each receptor will be sampling a big part of the image and there will be gaps in information between the receptors and, consequently, most of the detail of the image will remain unresolved. In contrast, if the receptors are small and closely packed together (while optically isolated from neighbouring receptors), then the image is sampled in more detail, and it will have a higher spatial acuity (Land 1981, 1985).

High spatial resolution is however, very demanding in terms of light (Warrant & McIntyre 1993). Given the severe trade off between resolution and sensitivity (i.e., resolution improving as the ratio of receptor diameter to focal length decreases, and sensitivity improving as the same ratio increases), this usually implies that an eye capable of producing high-resolution

images is not very sensitive to light. If an improvement in either resolution or sensitivity is required without sacrificing the other, then eye size must increase (Land 1981, 1985).

Although capturing sufficient light is not usually a problem for most diurnal animals, it can be a serious one for nocturnal animals or for those living in dimly lit habitats. As light levels fall so too does the reliability of vision. This is because the number of photons reaching individual receptors at low light levels is very small, creating a statistical uncertainty associated with the random nature of photon arrivals on the retina (Land 1981, 1985, Laughlin 1990, Warrant & McIntyre 1993). This uncertainty, which increases as the light levels fall, leads to a loss in the reliability of intensity measurements, and thereby the eye's ability to distinguish contrast details is also greatly diminished; ultimately, black cannot be distinguished from white (Land 1985, Warrant 1999, Land & Nilsson 2002).

The only way to overcome this problem is to increase photon capture (i.e., sensitivity). Several solutions have evolved in the natural world to accomplish this. Optically, an animal can develop: 1) wider pupils; 2) wider photoreceptors; 3) lenses with shorter focal lengths; or 4) a tapetum (i.e., a light reflecting structure inside the eye that gives the retina a second chance of capturing the photons missed on the first pass) (Land 1981, Warrant 1999, Land & Nilsson 2002). Neurally, photon capture can be improved by summing photons in space, through the coupling of neighbouring visual channels (spatial summation); or by summing photons in time (temporal summation), extending the time (integration time) during which a sample of photons is counted by the visual system (Snyder 1977, Laughlin 1990, Warrant 1999, Land & Nilsson 2002).

Adaptations such as these are commonly found in deep-sea and nocturnal animals such as nocturnal tarsiers, owl monkeys, tunas, swordfishes, octopuses (Warrant 2004), owls and opossums (Land & Nilsson 2002), crabs (Doujak 1985), toads, beetles (Warrant 1999), bees (Warrant *et al* 1996, Greiner *et al* 2004), harvestman (Meyer-Rochow & Liddle, 1988) and spiders (Blest & Land 1977, Laughlin *et al* 1980).

Jumping spiders (Salticidae) are renowned for their visual discrimination abilities (Land 1981, Land & Fernald 1992, Land & Nilsson 2002, Harland *et al* 1999), identifying different prey types, predators, rivals and mates from considerable distances (Crane 1949, Drees 1952, Forster 1979, Jackson & Blest 1982, Jackson & Li 1998, Harland & Jackson 2001, Jackson *et al* 2005). This is achieved through the combined work of eight camera type eyes. Six small secondary eyes spaced around the cephalothorax, work mainly as motion detectors. The remaining two large forward-facing eyes, known as "principal eyes", are responsible for high-acuity vision (i.e., the eyes' ability to resolve detail). When a target is detected by the secondary

eyes this evokes a turning response, and the object of interest is brought into the field of view of the principal eyes, which then process the details (i.e., size, shape, orientation) of the object that is being viewed (Land 1969a,b, 1971, 1974).

Considered to be one of the most remarkable eyes of the entire animal kingdom (Warrant & McIntyre 1993), salticid principal eyes are adapted for high spatial acuity vision (Land 1985). Unlike insect eyes, each salticid principal eye consists of a single fixed, non-malleable corneal lens formed by the carapace and a layered retina. The retinae are movable in all three dimensions and thus compensate for the eye's narrow field of view (Land 1969b). Each retina is embedded in a dense matrix, in which there is a concave pit symmetrically centred on-axis at the distal end. The pit functions as a diverging lens, increasing the focal length of the system, and magnifying the image formed by the corneal lens. Together, the diverging lens and the corneal lens form a telephoto lens system (Williams & McIntyre 1980a,b) similar to that found in raptors (Snyder & Miller 1978) and chameleons (Land 1995). This design allows for image magnification while avoiding an increase in the distance between the corneal lens and the retina, an increase, which would be impossible to accommodate within the restricted cephalic space of a jumping spider (Williams & McIntyre 1980a,b).

The retina of the principal eyes is also highly specialised (for a detailed description see Harland & Jackson 2004). The retina is a boomerang-shaped structure, which in the central region of highest acuity (i.e., the fovea) is made up of four tiers of receptors (Land 1969a, Eakin & Brandenburger 1971). Of the four layers, only layer I, the farthest from the corneal lens, has a sufficiently ordered mosaic capable of sampling high spatial resolution images. In extreme cases, such as the case of Portia, it reaches the remarkable inter-receptor angle of 0.04º, a value only comparable to that of *Octopus* (0.011º) and the human eye (0.007º), and much better than that of any other animal similar in size to a salticid (Land 1981, Land 1985, Blest & Sigmund 1984, Land & Nilsson 2002). Although the function of the other retinal layers is still uncertain, evidence suggests that the remaining layers may have a role in the detection of the plane of polarization of light (layer IV), and in colour vision through the absorption of different light wavelengths by the different layers (Land 1969a, 1985, Blest *et al* 1981).

Remarkable variation in the organization of retinal mosaic's Layer I has been documented in two primitive salticid subfamilies, the Lyssomaninae and the Spartaeinae (Wanless 1984a, Maddison & Hedin 2003, Su *et al* 2007), in a series of studies from David Blest's laboratory (Blest & Price 1984, Blest & Sigmund 1984, 1985, Blest 1985a, 1987a,b, Blest *et al* 1990). The morphological progression found suggests a step-by-step increase in spatial acuity, from foveal mosaics composed of short photoreceptors, each equipped with two

rhabdomeres and arranged as a rhabdomeral network (found in more primitive species), to highly ordered arrays of photoreceptors, each bearing a single rhabdomere designed to function as a light guide (found in more "advanced" salticids).

Apparent adaptations to the low habitat illuminances have also been found in both salticid principal and secondary eyes. Although most jumping spiders live in open, brightly-lit habitats, some live and hunt for their prey in dimmer microhabitats, like the under surfaces of broad leaves in the forest, in leaf litter, under rocks and even in the internodes of fallen bamboo (Blest 1983, 1985a, Jackson & Hallas 1986, Zabka & Kovac 1996). The principal eyes of species from dimly lit habitats usually see the power of their diverging lens reduced, their rhabdomeres are usually much wider, and they usually lack hypodermal pigment stop (i.e., a ring of pigment of fixed diameter that surrounds the rear face of the corneal lens though to control the amount of light entering the eye) (Land 1969a, Blest 1985a).

As in principal eyes, the size of the receptors found in the retina of secondary eyes also seems to be related with habitat light levels. Species inhabiting densely shaded habitats usually have much wider rhabdomeres than species living in more exposed habitats (Blest 1983). For a spider living in dim light conditions the significance of this is obvious; despite their less demanding role in terms of spatial acuity, secondary eyes must be still be capable of detecting movement using whatever light there is available. Therefore, the more sensitive the secondary eyes are the better qualified they are to detect movement under dim light conditions.

My research has been on *Cyrba algerina,* a spartaeine (Salticidae) species that lives and hunts for prey under stones in xeric areas (Jackson 1990). This salticid is particularly interesting from an evolutionary perspective because the retinal ultrastructure of its principal eyes is considered to be less organised than that of typical ("advanced") salticid eyes, and it has been suggested that *C. algerina*'s principal eyes might represent an intermediate stage in the evolution of the high-acuity retinal mosaics of modern salticids (Blest *et al* 1990). Here I suggest an alternative, but not exclusive, hypothesis: by living in a microhabitat where light levels are low, *C. algerina*'s retinal ultrastructure may as well represent an adaptation to the low ambient light levels imposed by its microhabitat, being a case were sensitivity has been favoured at the expense of acuity.

Although previous work (Blest *et al* 1990) has provided some information regarding *C. algerina*'s principal-eye retinal mosaic, no work has been done on its optics nor on is anterior lateral (AL) retina. This paper provides new information on the optics and histology of the principal and anterior lateral eyes of *C. algerina*. The possible advantages of *C. algerina*'s eye design, considering this species microhabitat light levels and way of life, are discussed.

Methods

Optics

Measurement of focal length

The focal length of the corneal lens of the principal eyes of *C. algerina* was measured directly using the hanging drop method devised by Homann (1928) (for details see Land 1985). The spider's corneal lens was first dissected under 0.9% saline. Each optical tube was carefully cut with a fine scalpel blade, as close as possible to the back of the lens, and removed so as to expose the hypodermal pigment stop (i.e., halo). Because the halo is very fragile and is easily torn away during the dissection of the lens only seven individuals provided measurements. Measurements of the halo were done using a stereo microscope at 25X magnification. The halo was then carefully removed and each lens was suspended in a drop of saline hanging beneath a slide coverslip, so that the corneal lens was in contact with air and the rear face of the lens was immersed in saline. The preparation was then positioned on the stage of a compound microscope to which the condenser had been removed. An arrow of known size (O) printed onto a card was then placed at a known distance (u) beneath the spider's lens, and the size of the image (I) formed by the spider's eye was determined using a 40x objective and an eyepiece graticule (Blest & Land 1977) (Fig. 1).

Histology

Spiders were immobilised with CO_2. The legs, palps and abdomen were removed while immersed in the primary fixative solution (2.5% glutaraldehyde, 0.1 M sodium cacodylate and 0.09M sucrose, adjusted to pH 7.3) (Blest *et al* 1988). To allow penetration of the fixative with minimal disturbance of tissues, small slits were made with a fine scalpel blade into the sides and base of the spider's cephalothorax and the specimen stored overnight at 4º C in fresh fixative solution.

The sternum, maxillae and the posterior end of the cephalothorax were removed the following morning. A "window" was then cut in the dorsal side of the cephalothorax, just above the eye tubes, to expose them to the fixative and ensure adequate fixation. Contrary to previous work (Blest & Sigmund 1984, Blest 1985b), the eye tubes were left *in situ* to avoid distortion. The tissues were then stored at 4º C for 2 hours in fresh fixative solution.

After being washed in buffer (3x 10-min washes at room temperature), the samples were post-fixed in a 1% buffered osmium tetraoxide solution at 4º C for 2 hours. They were then washed in distilled water (3x 10-min washes at room temperature) and dehydrated through an ethanol series (50%, 70% and 80% for 20 min each, and 90%, 95%, 100%, 100% for 15 min

each). After a final dehydration in 100% acetone (3x 15-min each), the tissues were infiltrated with Spurr's resin on a rotator, via an acetone/resin series: 1:1 (2 h), 1:3 (4 h), 1:7 (overnight) and finally cured overnight at 60º C.

Using a Leica Ultracut UCT ultramicrotome, longitudinal and transversal thick sections (1-2 μm) were cut and then stained with 1% toluidine blue (in 1% borax). Sections were then viewed using a Zeiss Axioskop 2 MOT light microscope, and images captured using a Zeiss AxioCam HRc CCD camera and AxioVision 3.1 software at a resolution of 1300 x 1030 pixels. Best estimates of the radii of curvature of the diverging component of the telephoto system were obtained from these sections using Image Pro Plus v 4.5 (Media Cybernetics, Inc.) and from prints.

Transverse ultra-thin sections (80-100 nm) of the retina were also cut and then stained with 5% uranyl acetate and Sato's triple lead citrate and examined under a Hitachi H-600 transmission electron microscope (TEM). All TEM sections illustrated are as near to true transverse or longitudinal as possible.

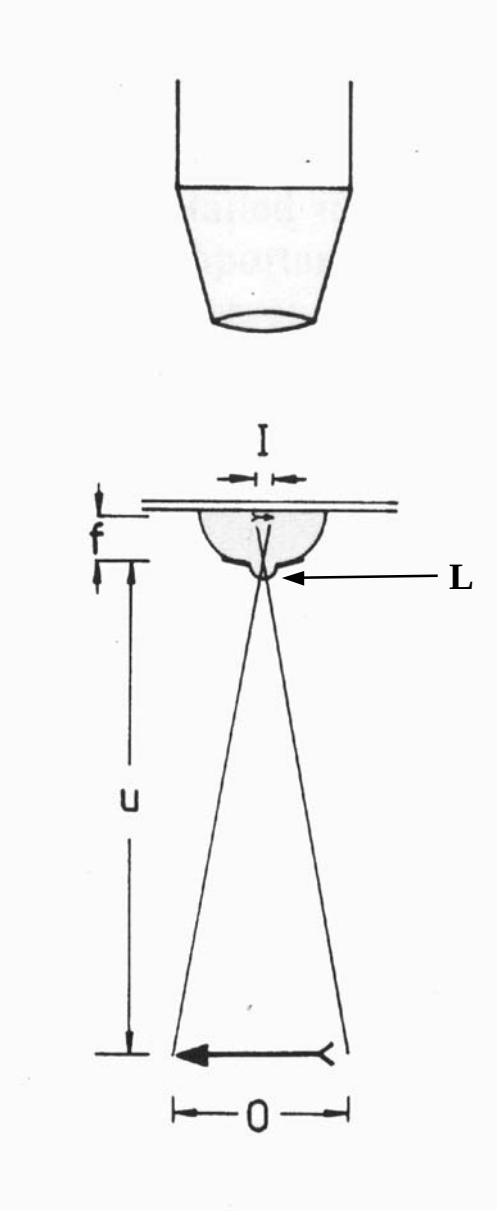

Figure 1. Homann's hanging-drop method for measuring focal length (from Land 1985). L- lens, I - image of object, O - object of known size, u - known distance, f - focal length (see text for details).

Results

Principal eye

Aperture of corneal lens

Although the size of the corneal lens among the individuals measured varied from 450 to 530 μm (mean ± SD: 487.7 ± 37.4 μm, N=7), the pigment stop was always 54 μm wide (mean ± SD: 54 ± 0.00 μm, N=7). If the extent of the pigment stop is taken as correct, a reduction in the effective aperture of the principal eye between 37-42% (mean ± SD: 39 ± 2.67 %, N= 7) should occur.

Focal length of corneal lens

The focal length of *C. algerina*'s principal corneal lens was calculated using the following equation:

$$f = \frac{I.u}{O} \qquad (1)$$

where I is the size of the image formed by the spider's eye, O is the size of an object placed at a distance u. An average focal length of 612 μm (N= 6, SD ± 32.8 μm) was obtained for *C. algerina*'s AM corneal lens using Homann's (1928) hanging drop method.

Magnification of the diverging component

The power of the corneal lens is increased by the diverging component of the telephoto system (pit), an interface located between the fluid-filled anterior chamber of the eye and the retinal matrix (Fig. 2). The magnification achieved by the diverging lens is inversely correlated with the radius of curvature of its apex; the smaller the radius of curvature, the greater the magnification afforded by the diverging lens (Williams & McIntyre 1980b). The magnification factor afforded by the diverging lens was calculated using the following equation (Williams and McIntyre 1980b):

$$M = \left[1 + \frac{P_P\left(1 - d' P_L\right)}{P_L}\right]^{-1} \qquad (2)$$

where P_P is the power of the diverging lens (dioptres), P_L is the power of the corneal lens, and d' is the distance in image space (d'=d/1.336), where d is the distance between the corneal lens (more precisely, its second principal plane) and the apex of the diverging lens.

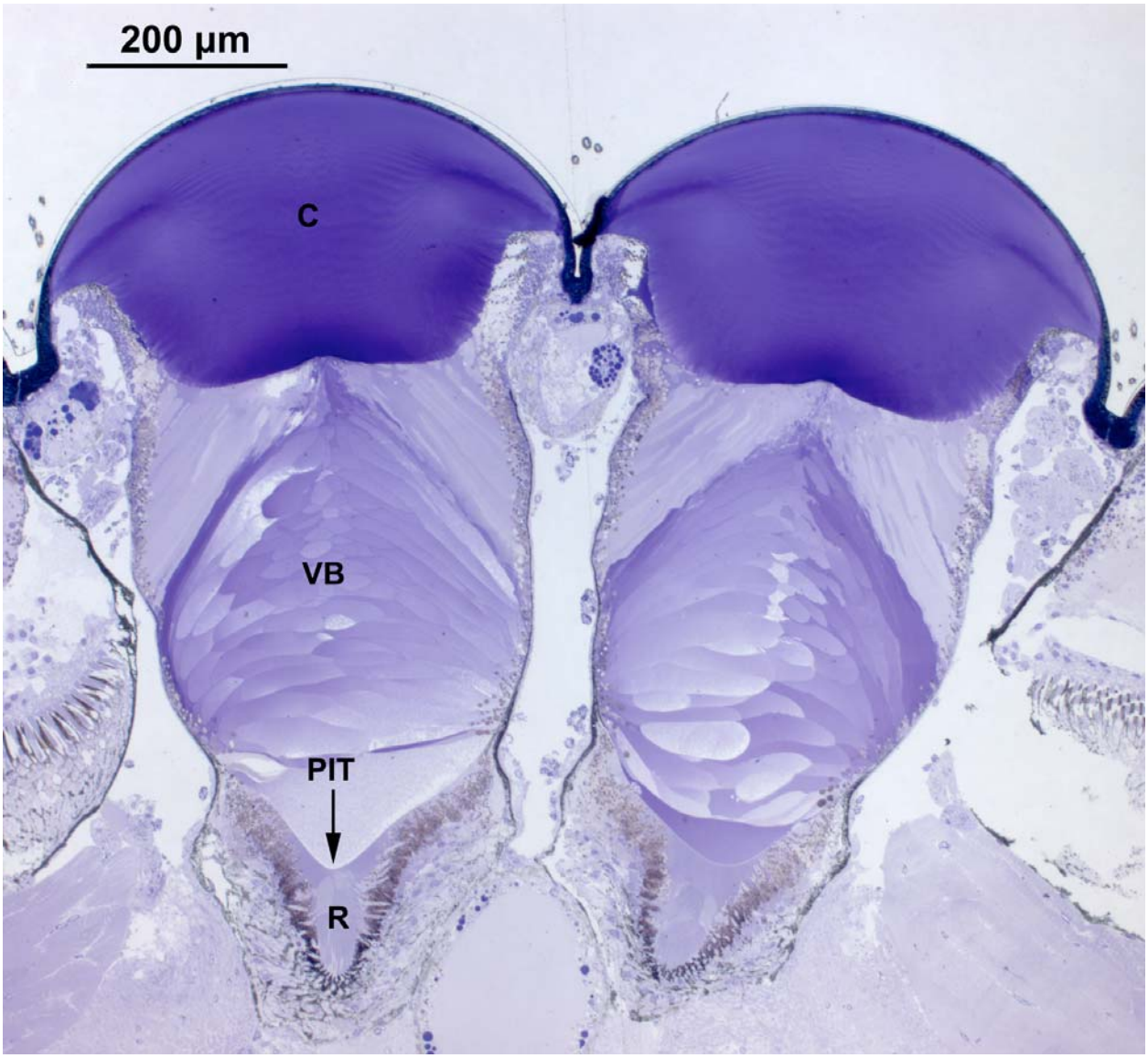

Figure 2. Light micrograph of longitudinal section of two entire anterior median eyes (AME) of *Cyrba algerina* showing telephoto arrangement. C - corneal lens; VB - vitreous body; PIT - diverging component of the telephoto system; R - four-layered retina.

The power of the diverging (P_P) and the corneal (P_L) lenses were calculated using the following equations (Blest 1985a):

$$P_P = \frac{n_M - n_A}{-r10^{-6}} \text{ dioptres,} \qquad (3)$$

$$P_L = \frac{1}{f} \text{ dioptres,} \qquad (4)$$

where n_M is the refractive index of the retinal matrix (c. 1.40 for *Portia fimbriata,* according with Williams & McIntyre 1980a), n_A is the refractive index of the material filling the anterior chamber of the eye, assumed to be that of saline (c. 1.336), r is the radii of curvature of the diverging lens, estimated from dorsal sections, and f is the focal length.

Although d is usually deduced from ophtalmoscopy (Williams & McIntyre 1980b) this technique was not available at the time. Alternatively, d was measured directly from longitudinal sections, under the assumption that the second principal plane of the corneal lens roughly coincides with the front of the lens, an assumption often true for complex lenses and multiple lens systems, as is the case of salticid principal eyes.

The focal length of the corneal lens of jumping spiders is directly related with the lens diameter (Blest 1985a). A relationship between the diameter of *C. algerina*'s corneal lens and its corresponding focal length was established through a linear regression, in order to predict the focal length for a corneal lens of a given diameter. This allowed the estimation of the focal length of the individuals used in histological work, as it is not possible to measure the focal length using the hanging drop method and perform histological work on the same individual (i.e., the hanging drop method requires the dissection of the corneal lens, rendering the specimen unsuitable for the type of histological work required). Given a corneal lens of 519 μm in diameter (measured from enlarged prints of dorsal sections), a focal length of 624 μm was estimated for an individual *C. algerina*.

The absence of external distinctive features that could be used as landmarks when aligning the specimen during sectioning, make it highly improbable that a correct alignment is actually achieved. The failure to do so inevitably introduces some level of error in the above calculations, as the distance between the pit and the principal lens (d) was taken from these sections (see above). To estimate the magnitude of the error caused by sectioning the specimen in an angle in relation to the optical axis, I calculated how much d would vary for a given sectioning angle (α angle). Considering that the principal lens in its central region is

hemispherical, the radius of the best fitting circle was determined and applied to the circle equation. The equation was then fed with "x" values to determine the corresponding "y" coordinates of a given point (x, y coordinates) in the lens. The distance (d_α) between the pit's apex and each point (x_α, y_α) was determined using the Pythagoras theorem, $x^2 + y^2 = hip^2$. I then calculated the associated α angle using the cosine and inverse cosine functions. d_α was then used to estimate the magnification afforded by the pit for a given alpha error and its effect in the magnification afforded by the pit and in the focal length of the telephoto system (Fig. 3 & 4).

The focal length *C. algerina*'s corneal lens alone is 624 μm. With the aid of the pit the power of the corneal lens is increased in about 1.29 times, providing the spider with a total focal length of 805 μm. If we assume a maximum alignment error of 5º in relation to the optical axis, the magnification factor provided by the pit is reduced to about 1.26 times, leading to a total focal length of 784 μm. As illustrated by Figures 3 and 4, unless the sectioning angle is especially large (i.e., an α error larger than 5º), the distance between the pit and the corneal lens (*d*), does not significantly affect the magnification afforded by the pit or the focal length of the telephoto system.

F-number

F-numbers (or F-stop) are a useful way of comparing the light gathering abilities of different eyes (Warrant & McIntyre 1991). The F-number of *C. algerina*'s lens system was calculated by dividing the focal length of the lens system by the aperture of the corneal lens. An F-number of 1.55 (or 1.95 if the presence of the pigment stop is taken into account) was obtained for *C. algerina.*

Retinal illuminance

Retinal illuminance $(A / f_{eq})^2$ depends both on the size of the AM eye entrance pupil (*A*) and on the focal length of the telephoto system (f_{eq}) (Blest & Land 1977, Blest 1985b). If the presence of the pigment stop is ignored (i.e., if the aperture is equal to the diameter of the lens), the retinal illuminance of *C. algerina*'s principal eye is about 0.233 (or 0.245 if we assume a 5º deviation from the optical axis during sectioning). If the presence of the pigment stop is taken into account, the effective aperture of the eye becomes considerably smaller and, consequently, the retinal illuminance of *C. algerina*'s principal eye is reduced to about 0.147 (or 0.155 if we assume a 5º deviation from the optical axis during sectioning).

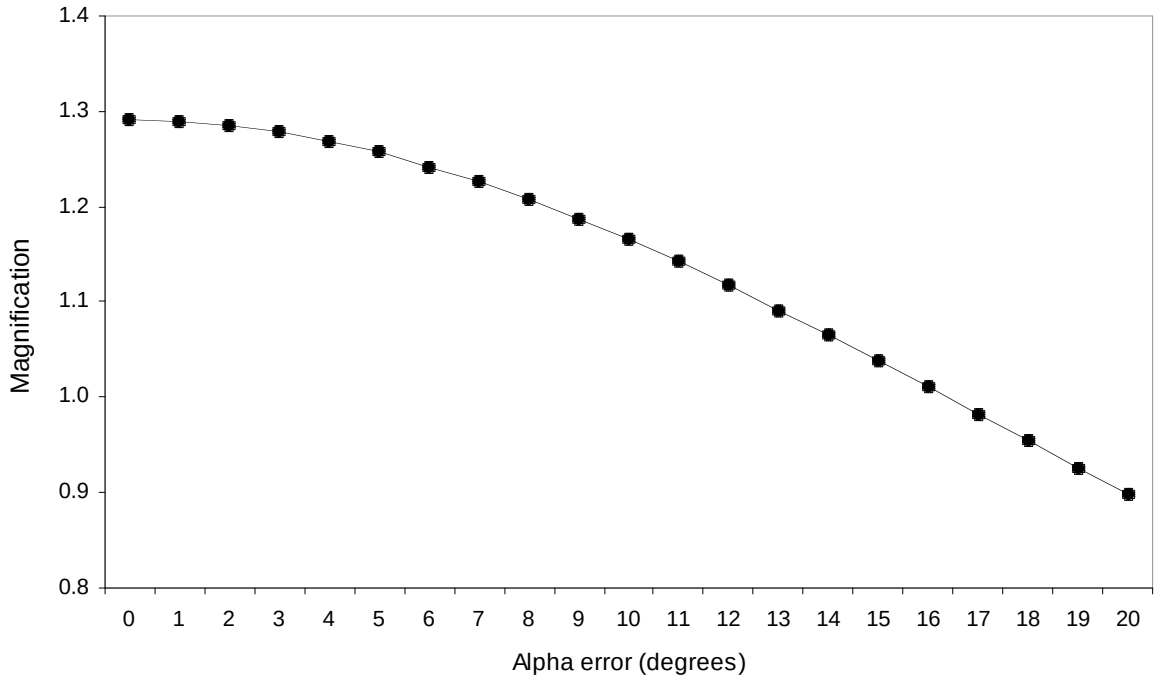

Figure 3. Effect of sectioning a specimen in an angle (alpha error) on magnification afforded by the diverging component of *Cyrba algerina*'s anterior median eye.

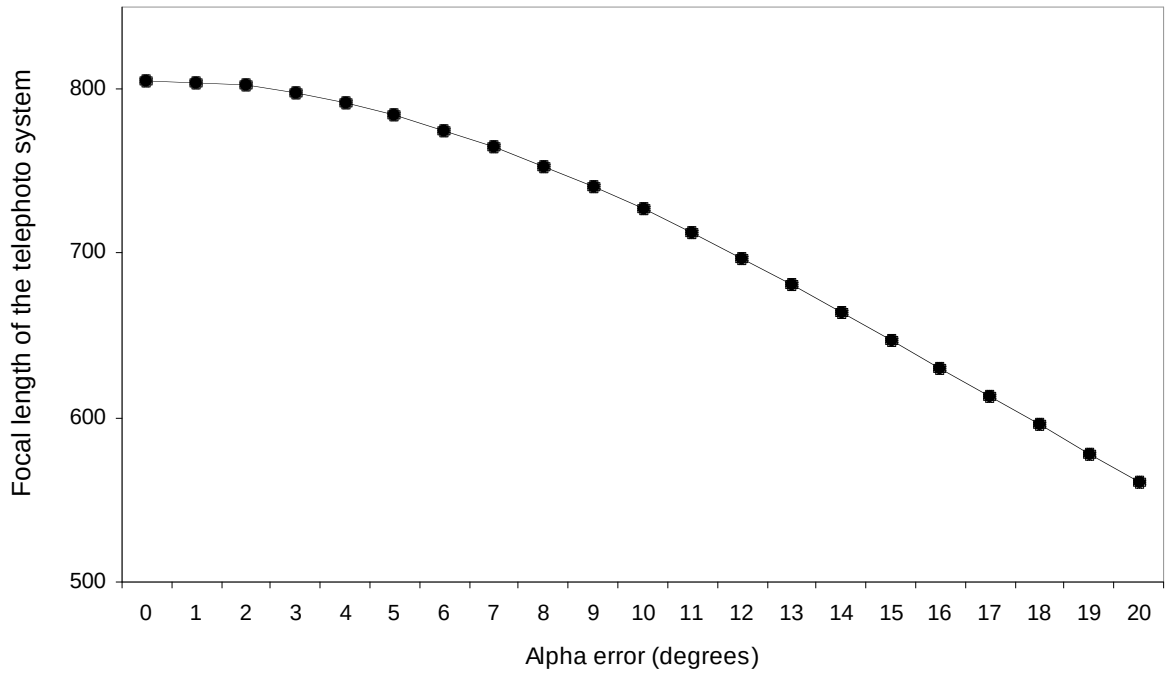

Figure 4. Effect of sectioning a specimen in an angle (alpha error) on the focal length of *Cyrba algerina*'s anterior median eye telephoto system.

Ultrastructure of the principal eye retina

C. algerina's layer I has been previously described by Blest *et al* (1990). A summary of their findings, as well as some additional details and images, are provided below.

According to Land *et al* (1990) *C. algerina*'s anterior median retina is composed of four layers of receptors arranged along the optical axis, similarly to that of other jumping spiders. Light reaching the eye must first pass through layers IV, III, and II before reaching layer I (Fig. 5), the only layer capable of producing images of high spatial acuity (Fig. 6). *C. algerina*'s layer I retinal mosaic is composed of 13 vertical rows of receptors, each bearing two rhabdomeres throughout the entire foveal region The rhabdomeres of adjacent receptors are contiguous forming shared light guides (i.e., twin rhabdomere arrangement), which should lead to optical pooling and consequent deterioration of image resolution (Fig 7). The outer rhabdomeres of the first three receptive segments on the outer side of the mosaic (fovea) are, however, very short, implying that its contribution to the pooled photon flux should be relatively small (Blest *et al* 1990).

Resolution of principal eye

Spatial acuity is directly related with the fineness of the retinal mosaic, the coarser the mosaic the worse the resolution (Land 1981, 1985). The inter-receptor angle ($\Delta\Phi$) subtended at the nodal point of the eye by an adjacent pair of receptors was used as a measure of spatial acuity (Land 1985):

$$\Delta\phi = \frac{\mathrm{d_{cc}}}{\mathrm{f}}, \qquad (5)$$

where d_{cc} is the centre-to-centre spacing of the retinal receptors, (equivalent to receptor width, as spider receptors are usually contiguous), and f is the focal length of the eye.

Measurements were taken from prints made from transverse ultra-thin sections (Fig. 7). Unfortunately, because I was not able to obtain a section illustrating the retina's entire foveal region, it is not possible to be sure of the exact location of the region illustrated on Figure 7. Therefore, the measurements here presented might be slightly different than those in the foveal region.

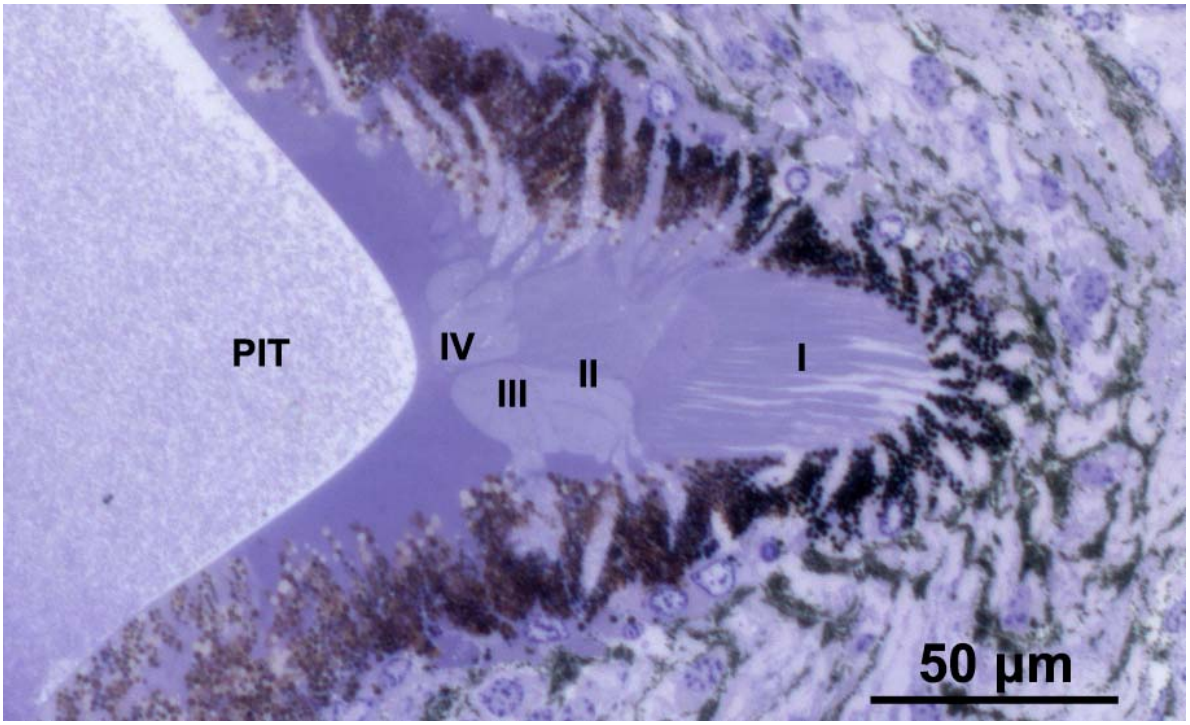

Figure 5. Light micrograph of longitudinal section across *Cyrba* a*lgerina*'s anterior median retina showing four layers of receptors (I, II, III and IV). PIT - diverging component of the telephoto system.

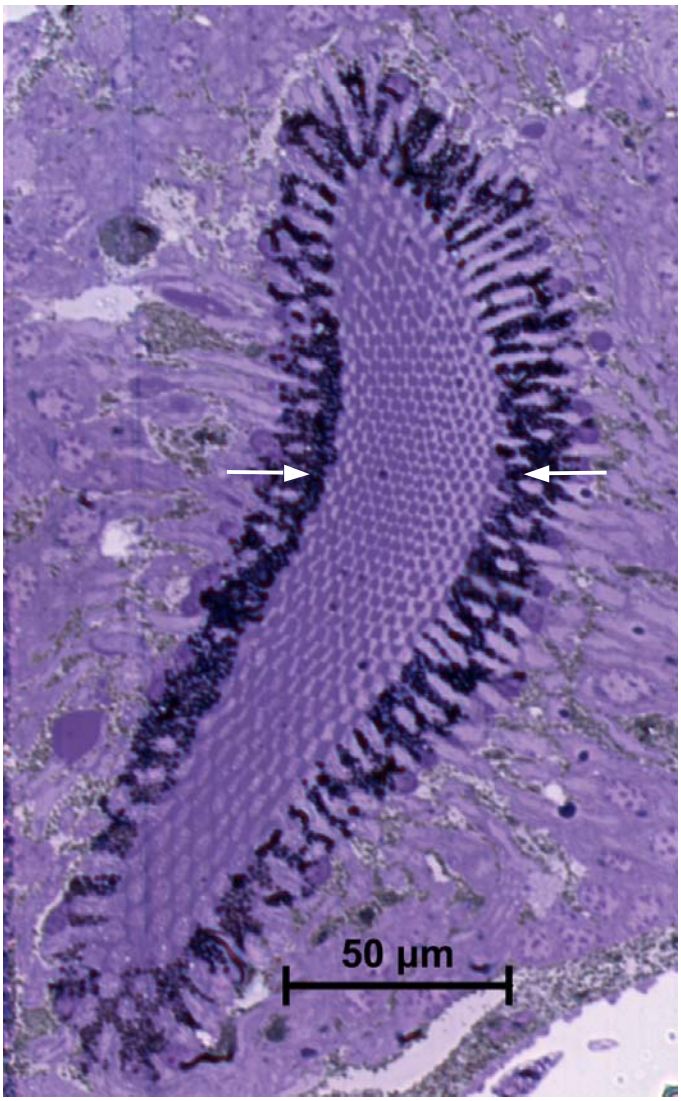

Figure 6. Light micrograph of transverse section through distal foveal region of Layer I of *Cyrba algerina*'s anterior median retina illustrating its boomerang shape. Section taken some distance proximally from tips of rhabdomeres. All 13 receptive segments represented.

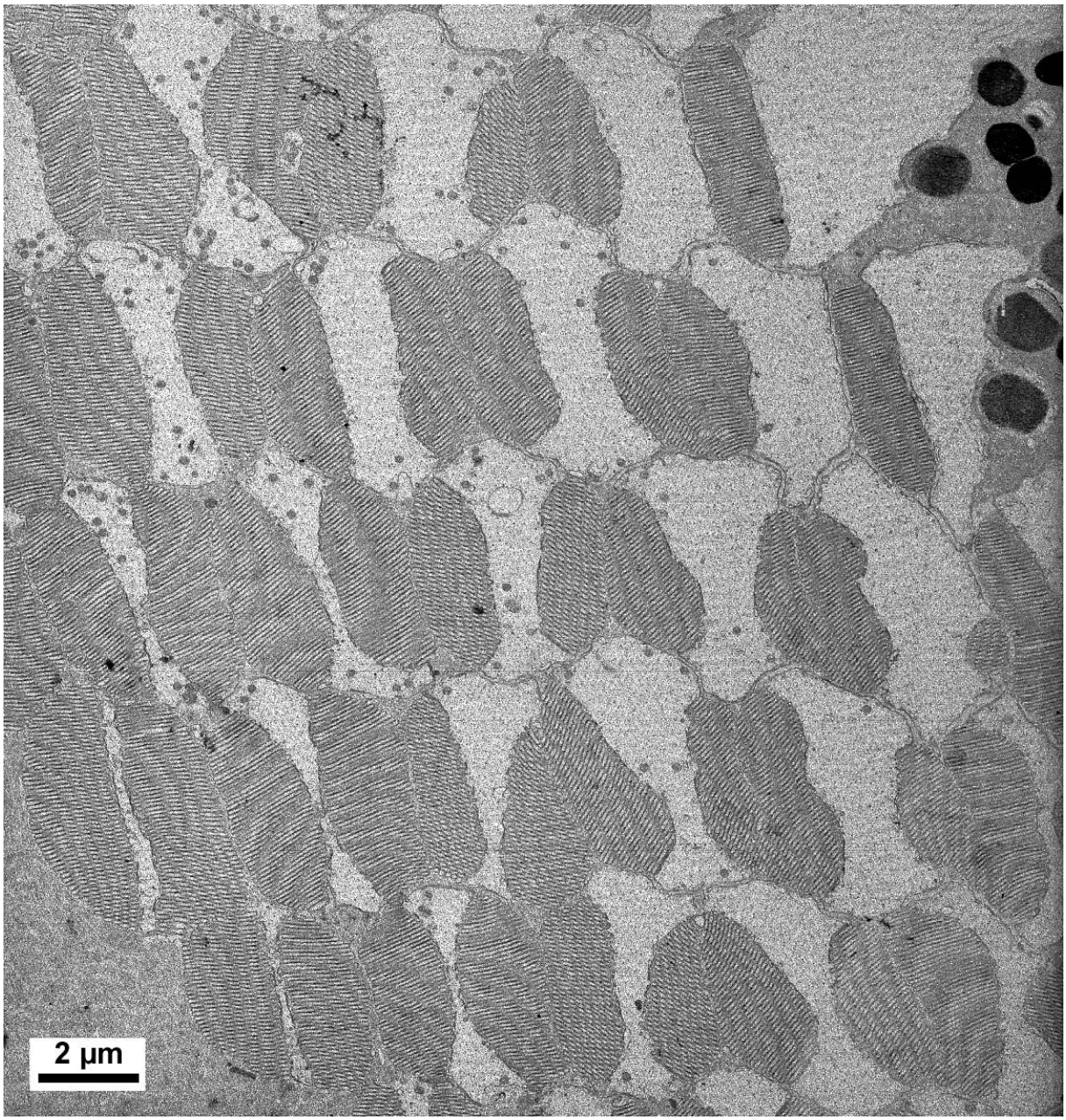

Figure 7. Ultra-thin transverse section through foveal region of Layer I of *Cyrba algerina* illustrating the twin rhabdomere arrangement of principal eye retinal mosaic. x 3500.

Because neighbouring rhabdomeres are contiguous at their tips, most of the rhabdomeres should behave as shared light guides (i.e., they should be treated as a single, but wider rhabdomere as opposed to two independent rhabdomeres (Blest *et al* 1990)). The minimum short and long diameters of twin rhabdomeres were c. 2.3 and 2.9 μm, respectively. Following Eqn. (5), and given a minimal rhabdomere separation of c. 2.9 μm, *C. algerina*'s anterior median eye should achieve a spatial acuity of about 0.21º, equivalent to 12.4 arc min in this particular region of the retina.

Length of rhabdomeres

An additional detail is provided regarding the length of layer I rhabdomeres. Although an accurate estimation of Layer I receptor length at the fovea was impossible to obtain, it should be reasonable to say from Figure 5 that *C. algerina*'s layer I receptors are no more than 40-45 μm long. Receptor length has a direct influence on the proportion of photons a rhabdomere is able to absorb, the longer the rhabdomere the more photons it will capture. Exactly how much light *C. algerina*'s rhabdomeres absorb is however, at least from our point of view, impossible to ascertain as the proportion of light absorbed by a rhabdomere also depends of its absorption coefficient (k), a parameter that has never been calculated for spiders, and that seems to vary among animal groups (Land 1981).

Anterior lateral eye

Salticid anterior lateral (AL) eyes are simple ocelli whose lenses provide images to a single layer of receptors, arranged in a single-layered bowl-like retina (Fig. 8) (Eakin & Brandenburger 1971). The ultrastructure of the anterior lateral retina of *C. algerina* consisted of well-separated receptors, each made up of two rhabdomeres, with oval transverse profiles. Each receptor was enclosed by two processes of non-pigmented glia, completely devoid of microtubules, which in turn were ensheathed by four pigmented glial processes filled with pigment granules (Fig. 9). Diameter of pigment granules was c. 0.5 μm. Short and long diameters of rhabdomeres varied between 1.4-2.1 μm and 3.4-3.9 μm, respectively.

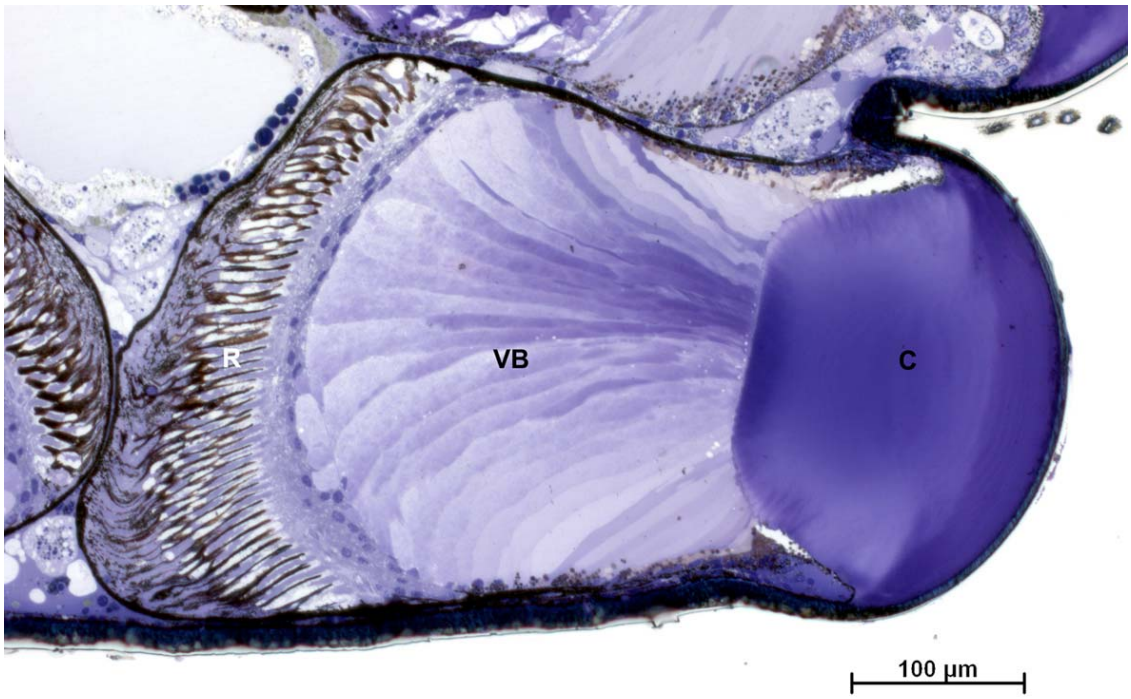

Figure 8. Light micrograph of longitudinal section of *Cyrba algerina*'s secondary anterior lateral eye (ALE). C - corneal lens of ALE; VB- vitreous body; R - single layered bowl-shaped retina.

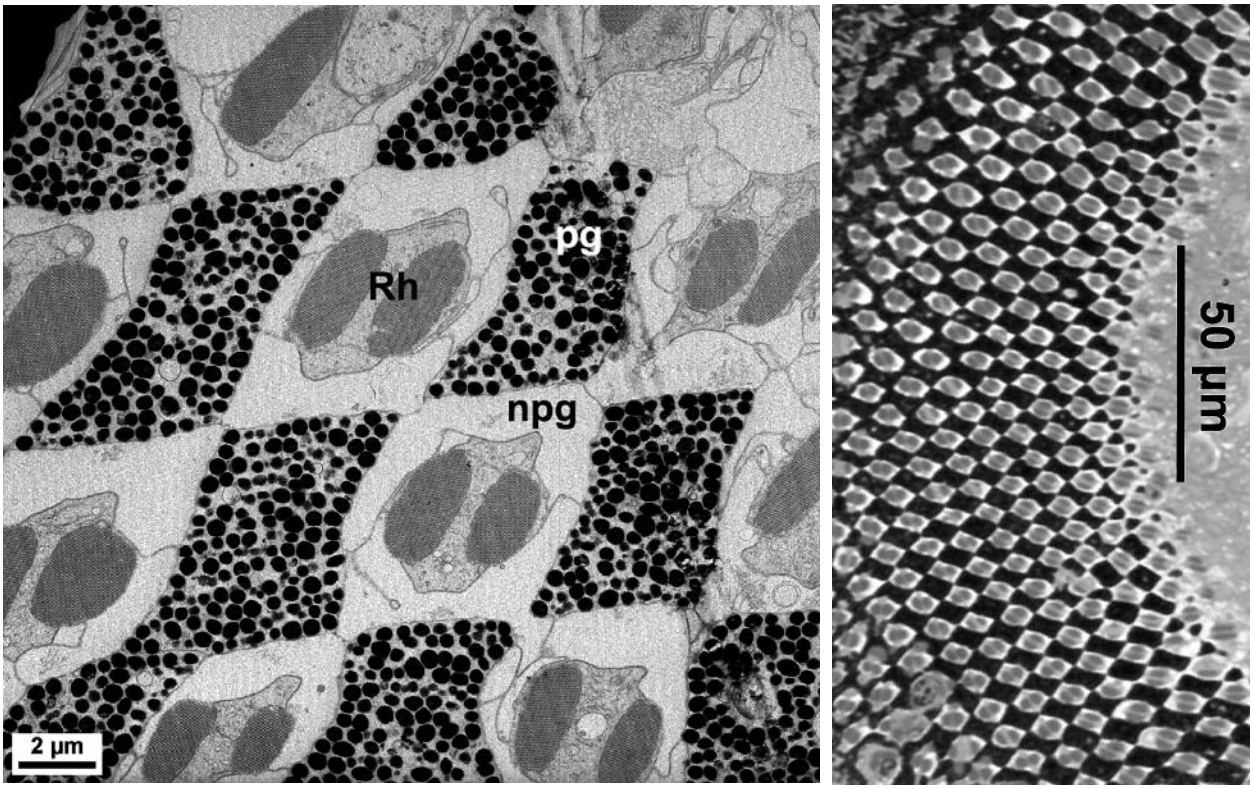

Figure 9 A, B. Transverse sections of *Cyrba algerina*'s anterior lateral eye (ALE). **A** Receptors composed of two rhabdomeres (Rh) flanked by non-pigmented glia processes (npg), surrounded by four processes of pigmented glia containing large pigment granules (pg) x 3500. **B** Light micrograph overview of ALE retina.

Discussion

Optics

F-numbers (also known as F-stops) are a useful way of comparing the light gathering abilities of different eyes. The lower the F-number the more light an eye is able to collect, species from dim habitats usually having eyes with smaller F-numbers than those living in bright habitats (Warrant & McIntyre 1991). However, in most salticids, both these parameters remain a function of spider size, independently of habitat light levels, F-numbers being around 1.80 (if the effects of the pigment stop are ignored) (Blest 1985a). A few species seem to be an exception to this rule; *Portia fimbriata* and *Phidippus johnsoni* have F-stops of 2.4 and 2.0, respectively (Warrant & McIntyre 1991). The same appears to be true for *C. algerina*, this species having an F-stop of 1.55 (if the presence of the pigment stop is ignored).

Although the optical role of the hypodermal pigment stop is still unclear, Blest (1985a) suggested it was a way of controlling the effective aperture of the lens, similarly to the pupil in our own eyes, but of fixed diameter. Its presence seems to be related with habitat illuminance, species from shaded habitats, such as *Fluda princeps* and *Itata completa*, usually lacking pigment stop, apparently allowing the eye to capture as much incoming light as possible (Blest 1985a). Although this is advantageous in terms of sensitivity, wider apertures may lead to spherical aberration and, therefore, deteriorate image quality. In *C. algerina* the presence of pigment stop reduces the area of the corneal lens (when looking through it) in about 39%. Similar values were found in *Plexippus validus* (Blest *et al* 1981), *Phiale magnifica* and *Jollas geniculatus* (using data provided by Blest 1985a). However, these species inhabit open space habitats, which are considerably brighter than *C. algerina*'s. If the role of the pigment stop is indeed to control the amount of light entering the eye, then its presence in *C. algerina*'s is surprising; considering the low ambient light levels under which this species lives, one would expect pigment stop to be absent. However, if we take into account the eye's effective aperture, we find that although *F. princeps'* does not have pigment stop, the eye's effective aperture is still smaller than *C. algerina*'s. While very low light levels are known to constrain eye design, this is especially problematic for species with small eyes (in absolute terms) (Blest 1985a), as the amount of light captured is usually related with eye size (Land & Nilsson 2002).

The same reasoning does not, however, apply to *I. completa*; *I. completa*'s principal eyes are bigger than *C. algerina*'s and still they lack pigment stop. In this case, focal length could be the responsible parameter for its absence. Because focal length is directly related with eye size, the bigger the eye the longer its focal length (Blest 1985a). Since eyes with long focal lengths are more demanding in terms of light, *I. completa*'s may have abdicated of its pigment stop to

increase photon flux and, therefore, compensate for its longer focal length. *C. algerina*'s intermediate eye size and corresponding shorter focal length may thus excuse the presence of pigment stop.

The curvature of the diverging lens varies greatly among jumping spider species. In some species, such as in *F. princeps*', it is so slight that the pit will have a negligible power (1.09) (Blest 1985a). In others, such as *P. fimbriata*, the pit's curvature is such, that it increases the anatomical resolving power of the principal eye 1.54 times in the central region of the retina (Williams & McIntyre 1980a,b). *C. algerina*'s pit increases the power of the corneal lens in about 1.29 (1.26 if assuming an α error of 5º) of times, providing the spider with a telephoto lens system 805 μm long (784 μm if assuming an α error of 5º). Higher magnifications can be advantageous because they improve spatial resolution. However, increased magnification will have a negative effect in terms of retinal illuminance; the higher the magnification factor afforded by the pit, the lower the amount of light reaching the receptors (Blest 1985a). Magnification is, as a result, in direct competition with retinal illuminance. In *C. algerina*'s case, although the power afforded by its pit is lower than that afforded by that of open space species, such as *J. genicullatus* (1.39 x) and *Phiale magnifica* (1.45 x), its retinal illuminance is relatively higher than that achieved by any of the above species (0.147-0.155 compared to 0.126 and 0.056, respectively (Blest 1985a)).

The features of *C. algerina*'s anterior median eyes may be related with this species particular microhabitat (i.e., the undersides of stones). Although the light levels of this particular microhabitat have never been measured, it should be safe to assume that this species lives under considerably low ambient light levels. Additionally, compared to the wide-open spaces where ordinary salticids are usually found, *C. algerina*'s microhabitat is more restrictive in terms of space and, therefore, more limiting in terms of the spider's range of view. In other words, there is probably no need for *C. algerina* to see over great distances considering the dimensionality of its microhabitat. On the other hand, if having a low magnification is also an advantage in terms of the light gathering abilities of the eye, then favouring retinal illuminance over anatomical power seems to be a good compromise.

Histology

AME Layer I

Blest (1985a) proposed two hypotheses to explain the evolution of the salticid retina. In the first he argued that the layer I mosaic of *Spartaeus,* which is composed of both single and twin rhabdomeres, is a true intermediate stage in the evolution of a high-resolution principal eye from

a low-resolution precursor (as previously suggested by Blest & Sigmund 1985). In his second hypothesis, he proposed that optical pooling between receptors over part of the mosaic, observed in some primitive forms (eg. *Lyssomanes* and *Yaginumanis*), is an adaptation to dim light.

Blest dismissed the second hypothesis, as none of the advanced forms studied to date, including those living in shaded habitats, possess receptors with twin rhabdomeres in layer I. Such species, namely *I. completa* and *F. princeps*, have instead developed single, shorter (74 and 45 μm, respectively) and wider receptors (2.7 and 3.3 μm, respectively) (Blest 1985a).

His second hypothesis does, however, raise an interesting point: can the retinal ultrastructure of intermediate primitive forms, such as *C. algerina*, be beneficial in terms of sensitivity, considering the low light levels imposed by these species microhabitat? In other words, and in *C. algerina*'s case in particular, can *C. algerina*'s retinal arrangement, while representing an intermediate step in the evolution towards a high spatially-acute eye, provide the spider with a more sensitive eye?

In principle, the sum of the signals collected by a group of smaller receptors from a particular retinal area, should achieve a similar signal to noise ratio to that of a single larger receptor occupying the same retinal area (Laughlin *et al* 1980). *C. algerina*'s twin rhabdomeres should, therefore, work as single but wider receptors, providing the spider with a considerably less spatially-acute but potentially more sensitive eye and, therefore, more adequate to the light levels of its microhabitat. Although under low light levels it is better to have a single larger receptor, providing a single but reliable signal (as seen in *F. princeps* and *I. completa*), than to have a large number of smaller receptors, each providing a less reliable signal (Laughlin *et al* 1980), the later solution (i.e., twin rhabdomeres) seems to have became available to *C. algerina* in the course of evolution towards high spatially-acute vision. In other words, *C. algerina's* twin-rhabdomere arrangement, although not representing the optimal solution to collect light under dim light conditions, might have just provided the spider with the necessary light-gathering abilities to strive in this particular microhabitat.

P. fimbriata is unusual among jumping spiders. Although being a primitive species and a close relative of *C. algerina* (Wanless 1984a, Madison & Hedin 2003, Su *et al* 2007), *P. fimbriata* bears a highly organized retinal mosaic, similar to that of more advanced salticids. Composed by single, long (90 μm) and narrow (0.8 x 1.5 μm) rhabdomeres, arranged as light guides throughout the entire foveal retina (Williams & McIntyre 1980b), *P. fimbriata*'s retinal mosaic confers the principal eyes with the highest spatial acuity (0.04º) known in jumping spiders or in any other animals of comparable size (Land & Nilsson 2002). *P. fimbriata*'s retinal ultrastructure is, however, unlikely to represent an early condition in the evolution of the retinal

mosaic (Blest & Sigmund 1984). The origin of *P. fimbriata'*s astonishing visual capabilities is said to be related with its complex predatory behaviour (Blest & Sigmund 1984); *P. fimbriata* is an araneophagic predator, known to exhibit highly specialised predatory strategies over a large array of dangerous prey in an enormous variety of situations (Harland & Jackson 2004). *P. fimbriata*'s principal eyes have, however, lost considerable sensitivity to light as a result of their high-resolution construction (Warrant & McIntyre 1993).

C. algerina's considerably shorter and wider twin receptors, sustain a much lower spatial acuity (c. 0.21º) than that of *P. fimbriata*, only comparable to that found in salticids inhabiting shaded habitats, such as *I. completa* (0.10º) and *F. princeps* (0.28º) (Blest 1985a). In *C. algerina*'s case the selection pressure might have acted in the opposite direction, towards sensitivity rather than spatial acuity.

The low light levels imposed by *C. algerina*'s microhabitat, together with a severe trade-off between resolution and sensitivity probably rendered the evolution of a more spatially acute mosaic impossible. Similarly to *P. fimbriata*, *C. algerina* also takes spiders as prey (Jackson & Hallas 1986, Jackson 1990, Jackson & Li 1998, Guseinov *et al* 2004, see Chapters 3 & 4). *C. algerina*'s diet is, however, more entomophagous than that of *P. fimbriata*'s, with insects constituting a considerable part of this species diet (Chapter 2). Prey diversity in *C. algerina*'s microhabitat also seems to be lower, as well as less dangerous (Guseinov *et al* 2004, Chapter 4), than that encountered by *P. fimbriata*. Together, this should make visual discrimination of prey a less demanding task for *C. algerina* than for *P. fimbriata*.

Anterior lateral eyes

C. algerina's anterior lateral (AL) retinal arrangement is similar to that observed in *P. fimbriata*, except for the shape of the transverse profiles of rhabdomeres, which in *Portia* are rectangular and in *C. algerina* appear to be oval, as in more advanced salticids. The non-pigmented glial processes also seem to be organised in a less orderly manner, and four instead of six pigmented glial processes, as found in more advanced salticids, surround the non-pigmented glia (Eakin & Brandenburger 1971, Blest 1983, 1985b, 1987a). Although this arrangement is considered primitive if compared to that found in more advanced salticids, *C. algerina*'s AL retina might be a step further from other Spartaeines species. *C. algerina*'s pigmented glial processes contain a much higher number of pigment granules than that observed in *Yaginumanis*, in which pigment granules are totally absent (Blest 1985b), in *Spartaeus*, where only a few scattered granules are present (Blest 1987a), as well as in *P. fimbriata* (Blest 1985b). Higher numbers of pigment granules are advantageous because they provide the receptive segments a more effective

shielding from scattered light (Blest 1987a), allowing the spider to place a moving object more precisely in its visual field (Eakin & Brandenburger 1971).

According to Blest (1983), the structure of the secondary retina is also passible of adapting to habitat light levels, species inhabiting densely shaded habitats having much larger receptors than species living in more exposed habitats, such as *P. johnsoni*. Compared to those of *P. johnsoni* (Eakin & Brandenburger 1971) and *P. fimbriata* (Blest 1985b), *C. algerina*'s receptors are relatively larger. If indeed the hypothesis suggested by Blest (1983) is correct then, *C. algerina*'s AL retina might also show some adaptation to dim light (i.e., be more sensitive). Additional histological work is necessary to confirm this hypothesis.

Conclusion

C. algerina's principal eyes are known to lack well-ordered retinal mosaics, a necessary condition to achieve high spatially-acute vision. For that reason this species visual abilities have been always considered relatively poor when compared to more advanced jumping spiders. Although *C. algerina*'s principal retinal mosaic is less orderly arranged, and may indeed represent an intermediate form in the evolution towards a spatially-acute principal eye, the anatomical features of *C. algerina*'s principal eyes have always been strictly evaluated in terms of spatial acuity, without ever considering the spider's lifestyle or the light conditions available at its microhabitat.

Both the optical and histological data presented in this paper show that *C. algerina*'s principal eye, although more limited in terms of spatial acuity, should be more sensitive to light. If taken into context, *C. algerina's* visual capabilities no longer seem inadequate, poor or limited, but instead an example of a salticid where sensitivity seems to have been favoured over spatial acuity, allowing this species to minimise the constraints imposed by its particular microhabitat.

A closer examination of *C. algerina*'s anterior lateral eye retina is necessary, but the histological data presented suggest that this species' anterior lateral eyes might also show some adaptation to low ambient light levels.

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CHAPTER 9

Orientation and prey capture in dim light by *Cyrba algerina*, a jumping spider that lives under stones

Abstract

Jumping spiders are well known for being diurnal predators that actively pursue their prey in the open, using elaborate vision-mediated behaviour. Although the vision-dominated mode of life usually attributed to salticids would seem most suited for brightly lit environments, there are some salticid species that frequent more shaded habitats and capture their prey under low levels of ambient light. *Cyrba algerina* is a striking example, as its microhabitat is the dimly-lit spaces on the undersides of stones. Here I provide experimental evidence that *C. algerina* can, while relying solely on vision, detect and identify prey under dim light. *C. algerina* also proved to be an effective predator in light levels under which other salticids perform poorly. *C. algerina*'s behaviour suggests that this species may be using temporal summation (i.e., summing photons in time by extending the time photons are counted by the visual system) to improve its visual performance in dim light. Ability to perform under dim light may be an important factor in enabling *C. algerina* to occupy a niche not available to the majority of salticids.

Introduction

Salticids resemble many other animals (Endler 1991) by having predatory sequences characterised by six distinct stages: encounter, detection, identification, approach, subjugation and consumption (Foster 1982a). Detection and identification are of special interest when considering salticid eyes. Salticids have eight eyes. Six secondary eyes, positioned around the salticid's cephalothorax, act primarily as movement detectors, and provide the spider with a combined field-of-view of almost 360° (Forster 1979, Land 1985). After movement is detected in the surroundings, the salticid responds by swivelling its body, so as to orient the corneal lenses of its large forward-facing principal eyes on the object that was detected. The principal eyes will then acquire information about the object's identity, such as an object's size, orientation and distance away (Land 1969a, 1971, 1985; Forster 1985), and sometimes remarkably precise information concerning different types of prey, predators and conspecifics (Harland & Jackson 2004). If the object is identified as suitable prey, the salticid will then approach it with more or

less care, depending on the prey's type, size and activity, and attack it either by leaping or lunging at it (Jackson & Blest 1982, Harland *et al* 1999). After a successful attack, the last stage, consumption, usually follows.

The remarkably well-designed eyes (Land 1969a,b, Blest *et al* 1990, Warrant & McIntyre 1993) and elaborate vision-mediated behaviour (Jackson & Pollard 1996) for which jumping spiders (Salticidae) are renowned is often compared to that of cats (Land 1974, Harland & Jackson 2000). However, unlike cats, much of salticid prey-capture behaviour seems to happen under bright light. Cats (Felidae: lions, leopards, domestic cats and so forth), in contrast, tend to be more active in prey capture under dim light at night. Cats' eyes are also much larger than those of salticids, and large eyes can be highly advantageous, as the trade-off between sensitivity and resolution should be considerably less severe in large eyes than in smaller eyes (Land 1981) such as those of salticids. Even so, the principal eyes of some salticids achieve a slightly higher spatial resolution than the eyes of domestic cats (Land & Nilsson 2002). But the high spatial resolution of salticid eyes comes with a cost; salticid small eye size implies a loss in sensitivity (Land & Nilsson 2002). This consideration leads to an expectation that salticids will be primarily predators that frequent well-lit habitats, capturing prey during the daytime and out in the open.

Despite this expectation, there are nonetheless some salticid species that frequent dimly lit habitats, including leaf litter, the undersides of large leaves in dense forest and even in the internodes of fallen bamboo (Blest 1983, 1985, Jackson & Hallas 1986, Zabka & Kovac 1996). The salticid I consider here, *Cyrba algerina*, seems to be an especially striking example of a salticid that captures prey in a dimly-lit microhabitat. This is a salticid that lives in the spaces on the undersides of stones. Although we cannot rule out the possibility that, for *C. algerina*, most predatory events take place in the open, under lighting conditions more normal for a salticid, this seems unlikely because *C. algerina* has only rarely been seen in the open (see Chapter 2), and the prey that seem most important in *C. algerina*'s diet are also found primarily on the undersides of stones.

The aim of this Chapter is to do a preliminary study on *C. algerina*'s ability to detect and identify prey under low ambient light. This will include experiments designed to determine the minimum light level under which *C. algerina* can, while using sight alone, detect, identify and capture prey. I will also consider what may be one of the interesting consequences of having, for a salticid, unusual ability to perform under dim light: *C. algerina* may be especially capable of preying on more ordinary salticids when they take shelter under stones under dim light. For examining this possibility, I staged encounters between *C. algerina* and another salticid, *Evarcha*

culicivora, known to capture prey primarily in the open, but also known to shelter in dimly lit microhabitats when quiescent (Wesolowska & Jackson 2003).

Methods

General methods

All *C. algerina* individuals used were adult females from the Sintra population. Maintenance, rearing-cage design and terminology follow those of earlier studies (Jackson & Hallas 1986) and only modifications and critical details are given here. Animals were kept under a 12 h/ 12 h dark/light regime and fed a mixed diet of fruit flies (*Drosophila melanogaster*) and juvenile New Zealand nursery-web spiders (*Dolomedes minor*) every 5-7 days (for more details see Chapter 3). Hunger level was standardised by keeping each individual of *C. algerina* without prey for 5 days before testing.

Predation tests were staged in complete darkness (i.e., in the absence of visible light) to determine whether *C. algerina* was capable of capturing prey in the absence of visual cues from prey. Additionally, three types of tests were staged under dim light levels: 1) mirror display tests, where *C. algerina* had access to visual cues from its own mirror image; 2) orientation tests, during which *C. algerina* had access to optical cues from the prey, but no vibratory or chemical cues; and 3) predation tests, where *C. algerina* had full access to prey.

All experiments were conducted in a lightproof room. Except when testing spiders in complete darkness, illumination was supplied by a 20W halogen lamp (Mickson-Model MF6356 AppN19584 240V 50Hz (NZ 230V)) placed directly above the testing apparatus. Light was dimmed using neutral density filters (Marumi ND4 and ND8 filters) in different combinations (e.g., ND20 = 1 ND4 filter + 2 ND8 filters), placed directly below the source light. Experiments were carried under five light levels: full light (233.89 cd/m^2), ND20 (1.35 cd/m^2), ND24 (0.54 cd/m^2), ND28 (0.24 cd/m^2) and ND32 (0.11 cd/m^2) (Fig. 1). The choice of light intensities used was based on preliminary experiments, with light levels higher than that allowed by ND20 having no apparent effect on the spiders' behaviour (i.e., once light level was higher than this, the spider's behaviour was indistinguishable from its behaviour under full light). Reflected light was measured using an International Light IL 1400 radiometer (in integrated mode) over an extended period of time to average out the noise.

Before testing, all spiders were kept in the dark for 1 h, so that they could acclimatise to the dark. Recording was undertaken with an infrared-sensitive video camera (Sony DCR-TRV18E). The expressions "usually" or "often", "sometimes" or "occasionally", and "rarely" or infrequently" are used, respectively, for frequencies of 80% or more, 20-80%, and 20% or less.

After each test all plastic and glass parts of the apparatus used were cleaned with ethanol and then with water to remove potential chemical cues left by the spiders.

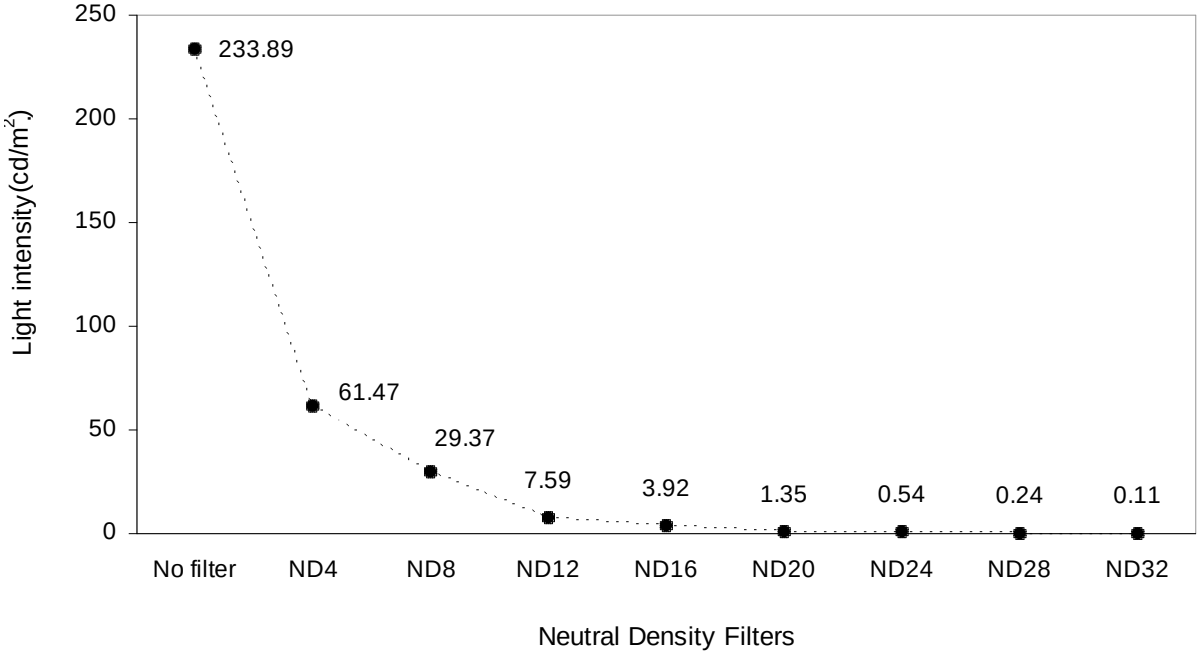

Figure 1. Light intensities used when testing under dim light. Different light intensities achieved by placing combinations of neutral density filters directly below the light source (e.g., ND 12= ND4 + ND8).

Mirror display

Basic testing methods were as in earlier studies (Jackson & Blest 1982, Harland *et al* 1999), except for the apparatus used. In earlier studies, the apparatus was a wooden ramp with a mirror positioned at the top end, but this was problematic when testing *C. algerina* given *C. algerina's* tendency to move under the ramp, and consequently invalidating many of the tests. This problem was solved by adopting an apparatus that kept the test spider enclosed with the mirror.

The apparatus consisted on a transparent plastic petri dish (140 mm in diameter), on to which a mirror (85 mm long and 15 mm high), positioned upright inside the petri dish, was glued. Entry was made via the "introduction hole", on the side of the petri dish, opposite to the mirror, so that a spider entering the petri dish immediately faced the mirror (Fig. 2). Before testing began, an individual of *C. algerina* was taken into a plastic tube (20 mm long 8 mm in diameter) and its two ends plugged with corks. After a 5-min acclimatisation period, one of the corks was removed and the end of the tube was fit in the introduction hole on the side of the petri dish. *C. algerina* usually walked spontaneously out of the tube and into the petri dish. However, if the test individual was still in the tube after 10 min, the other cork was removed and a soft brush was slowly inserted to entice *C. algerina* out into the petri dish. Testing began when *C. algerina* entered the apparatus.

A sheet of paper ruled from the mirror at 10 mm-intervals and placed under the base of the petri dish, allowed the determination of the distances from which the spider first displayed at the mirror. The distance from which the spider first displayed at the mirror and the spider's subsequent behaviour was recorded. Tests ended when *C. algerina* moved away from the mirror, either without stopping or fixating on the mirror (i.e., *C. algerina* held its body oriented perpendicularly to the mirror so that its principal eyes faced the mirror for at least 5 s), or after the test spider approached and displayed at the mirror.

Only tests where the spider fixated on the mirror were considered valid tests. Spiders that failed to fixate on the mirror were retested up to 2 times per day with 30 min intervals during 2 days. Fixating on the mirror was taken as evidence that the spider had detected the presence of an object. If a spider fixated on the mirror but did not display that was taken as evidence that the spider detected the presence of an object but failed to identify it has a conspecific female. Display at the mirror was considered evidence that the spider identified its mirror image as a conspecific female.

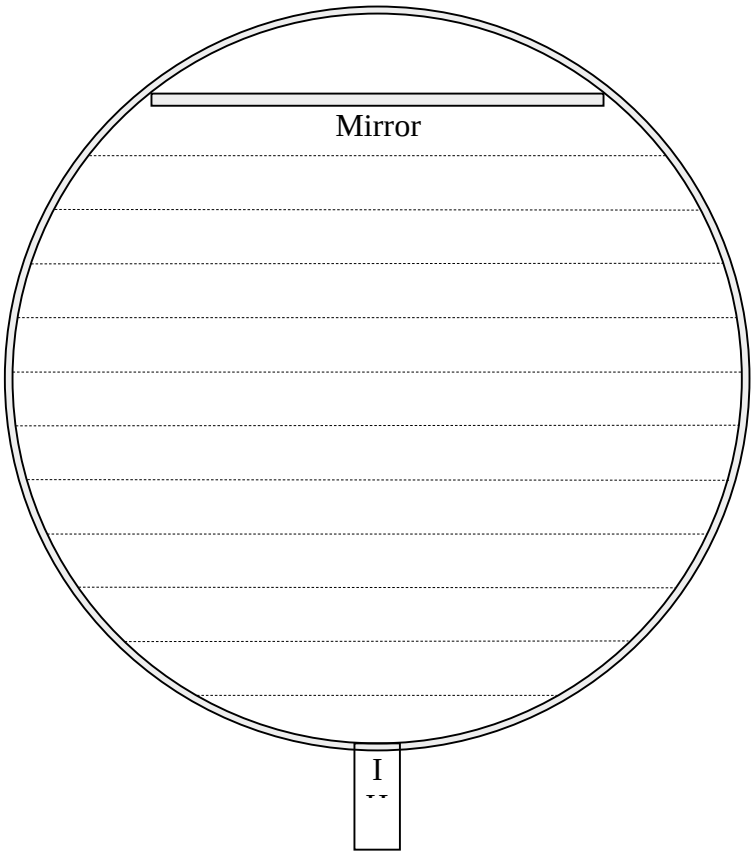

Figure 2. Mirror-display apparatus (viewed from above). *Cyrba algerina* entered apparatus through introduction hole (IH).

Display distance (i.e., the distance from the anterior margin of the spider's cephalothorax to the mirror) was doubled to account for the distance of the spider's virtual image in the mirror (see Harland *et al* 1999). The use of this technique allowed testing the animal's visual discrimination abilities with animated stimuli (i.e., its mirror image) while eliminating all non-optical cues and, therefore, guaranteeing that identification was based on optical cues alone. Furthermore, because the posture adopted by spiders during mirror display tests is unique to intraspecific encounters, the adoption of this posture indicated that a spider had identified the mirror image as a conspecific and not as any other object (e.g., prey or a non-conspecific salticid).

Orientation to prey

Using a specially designed apparatus, I evaluated *C. algerina*'s ability, when using vision alone, to detect and orient accurately towards live moving prey. The apparatus consisted on a petri dish (85 mm in diameter) with a circular hole (25 mm in diameter) in its centre, in the middle of which stood a test chamber (Fig. 3). The test chamber was a cylindrical, transparent glass container (22 mm in diameter, 15 mm high, similar in shape to the lid of a petri dish) fitted on a circular cork base. The cork worked simultaneously as a base for *C. algerina* to stand and as a stopper for the test chamber. The sides and the base of the petri dish were covered with white filter paper to provide contrast between the prey and the background. The filter paper covering the base was divided from the centre in 12 equal sectors of 30º each. This allowed the determination of *C. algerina*'s orientation in relation to the prey. To ensure that *C. algerina* did not use vibratory cues produced by the prey as it moved around the petri dish, the latter was isolated from the table by three pieces of cork glued to its base. The cork base of the test chamber provided additional isolation from vibrations.

As prey I used a common New Zealand wolf spider, *Lycosa* sp. (Lycosidae). When placed inside a petri dish the lycosid usually spent most of its time running along the sides of the petri dish, providing the test spider with constant and adequate visual stimulation. The lycosids were always similar in size to the test spider.

Before each test *C. algerina* was placed inside the glass container and covered with a piece of clean paper towel. The cork base was then carefully pushed inside the glass container until secure, making sure that the spider had enough space to move around comfortably. The paper towel provided contrast between the test spider and the base of the chamber necessary to allow recording under infrared light. After a 5-min acclimatisation period, the test chamber was then placed in the middle of the petri dish (in the hole) containing the prey, making sure that it

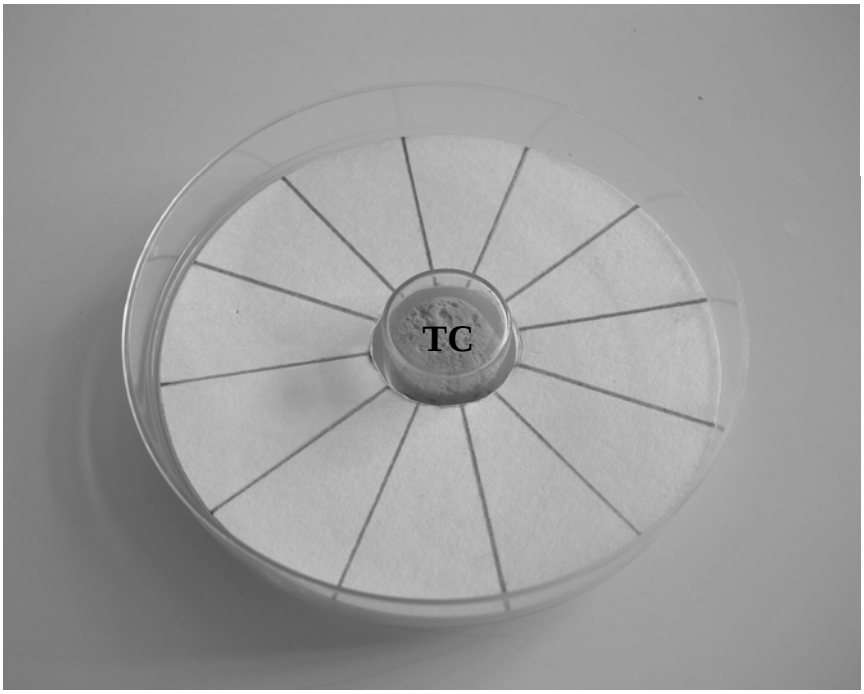

Figure 3. Orientation test apparatus. *Cyrba algerina* was placed inside testing chamber (TC) and its orientation towards *Lycosa* sp. recorded for 5 min. Filter paper covering base of apparatus divided from the centre in 12 equal sectors (30º each) to allow determination of *C. algerina*'s orientation in relation to prey.

was not contacting the petri dish. Tests began as soon as *C. algerina* first oriented to the prey and observations continued for 5 min. If *C. algerina* failed to orient at the prey after 5 min of being placed inside the test chamber, the test was aborted and the spider was tested a second time 30 min later or on the following day.

Predation in the dark

As prey I used two species of oecobiids (*Oecobius machadoi* and *O. amboseli*), two other spider species (*Trachyzelotes bardiae* (Gnaphosidae) and *D. minor* (Pisauridae)) and bristletails (*Ctenolepisma* sp.). The predatory tactics *C. algerina* adopted with these prey were previously assessed under full light (see Chapter 4). All prey, except for bristletails, were always smaller or similar to *C. algerina* in body size.

Testing was initiated by allowing an individual of *C. algerina* to enter an arena (diameter of petri dish, 85 mm) containing prey. *C. algerina* has a strong tendency to walk on the sides and edges of petri dishes and since oecobiids also seem to adopt the sides of petri dishes when building their nests, oecobiids were tested in a specific experimental arena so as to minimise the frequency with which *C. algerina* contacted the oecobiid's nest merely by chance (see Chapter 4 for more details). Entry was made via a plastic tube fitted on the side of the petri dish. The outer sides of the petri dish were covered with white paper to provide adequate contrast. Observation continued until *C. algerina* captured the prey or until 90 min had elapsed. No individual spiders were tested or used as potential prey more than once per day.

Predation in dim light

The methods used were similar to those described for staged encounters in the dark. As prey I used *D. minor*, the New Zealand nursery-web spider, and *E. culicivora*, a Kenyan salticid species.

Dolomedes minor

The decision to use this species as prey was based on *C. algerina*'s preference for spiders over insects as prey (Jackson & Li 1998), and on the species' availability (i.e., *D. minor* is a common spider in New Zealand and is easy to maintain in the laboratory). Testing was initiated by allowing an individual of *C. algerina* to enter a petri dish (85 mm in diameter) containing an individual *D. minor*. Entry was made via a plastic tube fitted on the side of the petri dish. The outer sides of the petri dish were covered with white paper to provide contrast.

Tests began as soon as *C. algerina* first oriented to the prey and observations continued for 30 min. or until prey capture occurred, whichever happened first. Spiders were tested under three light levels: No filter (i.e., full light), ND 24 and ND 28. All *D. minor* individuals used were always smaller than or similar to *C. algerina* in body size.

Evarcha culicivora

Evarcha culicivora is a salticid from Kenya, known for preying preferentially on mosquitoes (Jackson *et al* 2005). The choice to use of this species was related to its availability (cultures were already established in the laboratory), and to the fact that this species captures its prey primarily in the open, but when quiescent it seeks shelter in dimly lit microhabitats close to the ground, such as among grass or other vegetation at the base of tree trunks and lower reaches of the walls of houses (Wesolowska & Jackson 2003). This allowed me to investigate an interesting possibility: that *C. algerina* might be exploiting its unusual ability to perform under dim light to capture salticids that are seeking shelter in dimly lit habitats.

Encounters between *C. algerina* and *E. culicivora* were first staged under full light to assess how these two species interact under normal light conditions (i.e., when both salticids can see well). Additional encounters were then staged under dim light (ND24 (i.e., 0.54 cd/m^2)) to evaluate the effect of dim light on the behaviour of the two salticids.

Each *C. algerina* individual was tested separately with two sizes of *E. culicivora* individuals: small (c. half the body length of *C. algerina*), and medium (similar in size to *C. algerina*). Tests began as soon as *C. algerina* first oriented to the prey and observations continued for 30 min. or until captured occurred, whichever happened first. Only interactions in which at least one of the salticids stared at the other were considered. No individual spiders were tested or used as prey more than once per day.

Results

Mirror display

After entering the petri dish, *C. algerina* usually moved forward towards the mirror, then stopped and fixated on the mirror. Next *C. algerina* either moved away from the mirror or, most often, displayed at its mirror image by first erecting legs I & II (see Chapter 4). Spiders then approached the mirror and adopted the lateral-hunched posture. Some spiders approached the mirror in the lateral hunched legs posture while zigzag dancing, eventually being only a few millimetres away from the mirror. However, most often the spider approached the mirror by slowly moving forward without dancing. At this stage most spiders lunged or charged at the

mirror (i.e., spiders suddenly ran towards the mirror, but stopped before making contact) and then ran away.

This behaviour was observed in most individuals when under full light (no filter) as well as when under 1.35 cd/m^2. The number of spiders that displayed decreased steadily as the light level decreased (see Fig. 4 & 5), until only one (0.54 cd/m^2), and eventually none of the spiders displayed at the mirror (0.11 and 0.24 cd/m^2). The distance from which the spiders displayed at the mirror also decreased as light became dimmer (about 16% when under 1.35 cd/m^2, and about 80% when under 0.54 cd/m^2).

Orientation to prey

When under full light *C. algerina* usually oriented immediately and very accurately towards moving prey. However, if the prey was motionless *C. algerina* almost never oriented towards it. *C. algerina* then tracked the prey as it moved along the sides of the petri dish, adjusting direction and speed of swivelling according with the prey's movements. If the prey became motionless while being tracked, *C. algerina* usually stopped and faced the prey until it moved again. How long *C. algerina* faced the prey varied from a few seconds to as long as 3 min, after which *C. algerina* usually turned away.

There were several ways in which light level seemed to affect orientation and tracking of prey. The number of spiders that oriented and the frequency with which they oriented to the prey diminished markedly as the light became dimmer (see Fig. 6). Light levels also affected how long *C. algerina* stared at motionless prey (i.e., the dimmer the light, the less time the spider stood facing the prey). *C. algerina* faced motionless prey for as long as 3.5 min under full light, but for a maximum of only 5 s when light level was 0.11 cd/m^2. The accuracy with which *C. algerina* oriented towards prey decreased and response latency also became longer. When light level was 0.24 or 0.11 cd/m^2, spiders generally showed a discrepancy of c. 10º between the prey's location and their own final orientation, as well as a delay in time of response of c. 1-3 s (i.e., although *C. algerina* swivel its body immediately when under full light, *C. algerina* took 1-3 s before swivelling under dim light).

C. algerina also seemed to lose the ability to track the prey when under 0.11 cd/m^2; although still able to orient accurately towards the prey, *C. algerina* failed to track the prey that was moving around the petri dish.

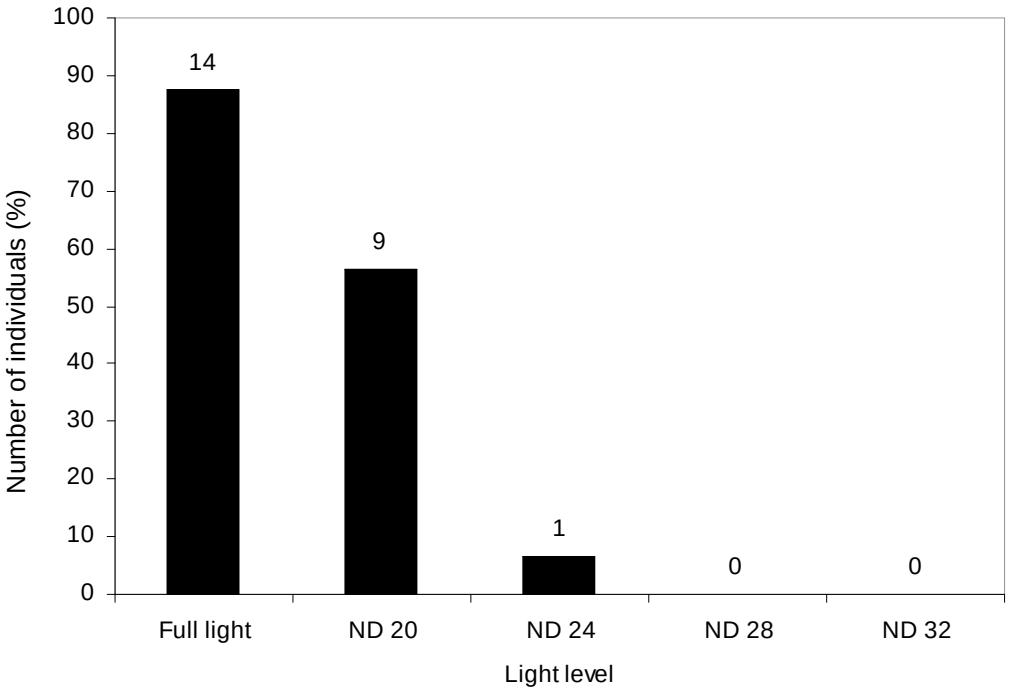

Figure 4. Percentage of *Cyrba algerina* individuals that displayed at mirror under different light levels: full light=233.89 cd/m^2, ND20=1.35 cd/m^2, ND24=0.54 cd/m^2, ND28=0.24 cd/m^2 and ND32=0.11 cd/m^2. Number of individuals that displayed at mirror under each light level given on top of bars (N=24).

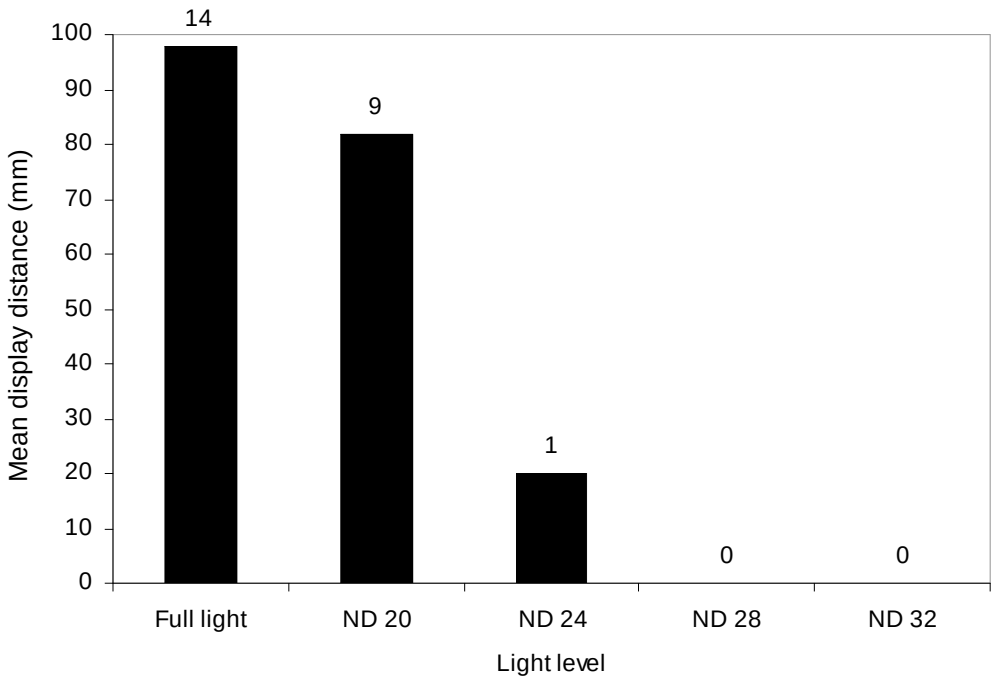

Figure 5. Mean mirror-display distances of *Cyrba algerina* under different light levels: full light=233.89 cd/m^2, ND20=1.35 cd/m^2, ND24=0.54 cd/m^2, ND28=0.24 cd/m^2 and ND32=0.11 cd/m^2. Number of individuals that displayed at mirror under each light level given on top of bars (N=24).

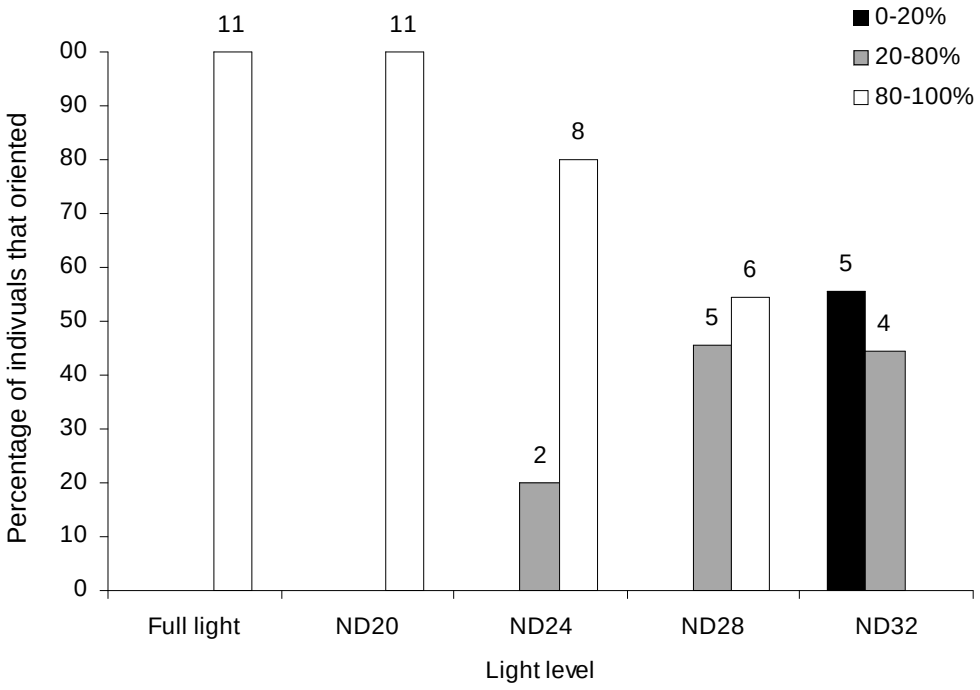

Figure 6. Percentage of *Cyrba algerina* individuals that oriented correctly (0-20%, 20-80% and 80-100% of times) towards *Lycosa* sp. under different light levels: full light=233.89 cd/m^2, ND20=1.35 cd/m^2, ND24=0.54 cd/m^2, ND28=0.24 cd/m^2 and ND32=0.11 cd/m^2. Number of individuals that oriented correctly under each light level given on top of bars (N=12).

Predation in the dark

C. algerina never captured any of the prey species used during predatory encounters staged in complete darkness. Each instance of *C. algerina* approaching a prey individual seemed to be accidental and, whenever it got close to the prey, *C. algerina* immediately moved away. *C. algerina* was never observed orienting, stalking, crouching or lunging at any prey species.

Predation in dim light

Dolomedes minor

As when under full light (see Chapter 4), most predatory sequences were initiated when *D. minor* started moving around the arena, but detection of prey was not as immediate, as when under full light. *C. algerina* did not always orient the first time *D. minor* moved about in the arena. Although *C. algerina* was still able to detect *D. minor*'s presence in the arena and orient towards it under dim light, the number of lunging events and how successful the lunges were decreased considerably as light level decreased (Fig. 7). *C. algerina* also took longer to approach *D. minor* (up to 5 min), stopping to face the prey for long periods of time in between locomotion boots.

When under the lowest light level used (0.24 cd/m^2) *C. algerina* only rarely captured *D. minor*. Most often *C. algerina* approached and stared at *D. minor* for a variable period of time (from 3 s up to 4 min), and then moved away without ever lunging. After this most *C. algerina* did not approach *D. minor* again. Occasionally, after facing *D. minor* from 2-3 body lengths away for a few seconds without approaching it at all, *C. algerina* simply moved away. Attacks on moving prey were never observed.

Evarcha culicivora

Interactions with small and medium sized *E. culicivora* were similar in most respects except that *C. algerina* lunged at small *E. culicivora* more frequently than at medium sized individuals. Capture success was similar with the two prey sizes (Fig. 8).

During typical interactions, as soon as *C. algerina* detected *E. culicivora* it immediately oriented towards it and started stalking *E. culicivora* around the arena. When close, *C. algerina* usually crouched and stared at the salticid for highly variable time periods, after which it usually pulled its legs I-III back and lunged. During these encounters *E. culicivora* never oriented towards *C. algerina*, not even when *C. algerina* was only a few mm away. *E. culicivora* seemed to be completely unaware of *C. algerina*'s presence. After an attack *E. culicivora* simply moved away but no other changes in behaviour were noticeable. After staring at *E. culicivora* for a while, *C. algerina* sometimes moved away without lunging at *E. culicivora*. This was more

common when *E. culicivora* and *C. algerina* were similar in body length (i.e., medium size *E. culicivora*) but also occurred with small *E. culicivora* individuals (Fig. 8).

On one occasion *C. algerina* was observed displaying at a small *E. culicivora* individual. *C. algerina* displayed by first erecting legs I & II and then adopted the lateral-hunched posture and zigzag danced (see above for more details), a display commonly used when displaying at conspecific females. *E. culicivora* did not display, or approached *C. algerina* and *C. algerina* eventually moved away without ever lunging at *E. culicivora*.

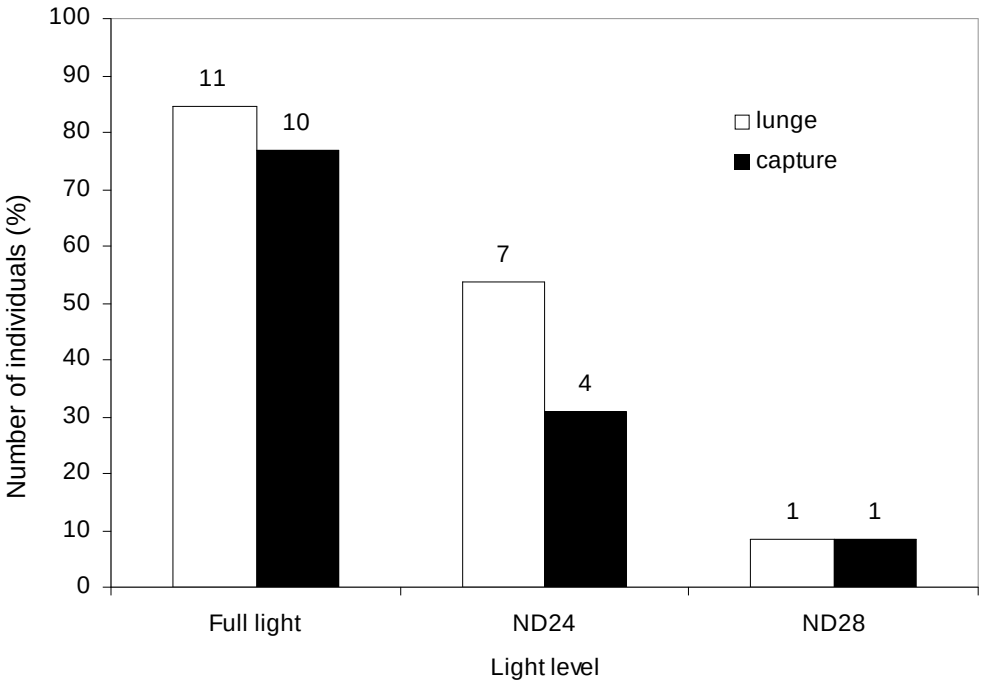

Figure 7. Percentage of *Cyrba algerina* individuals that lunged and captured *Dolomedes minor* during staged predatory encounters under different light levels: full light=233.89 cd/m^2, ND24=0.54 cd/m^2 and ND28=0.24 cd/m^2. Number of individuals that lunged and captured *D. minor* under each light level given on top of each bar (N=12).

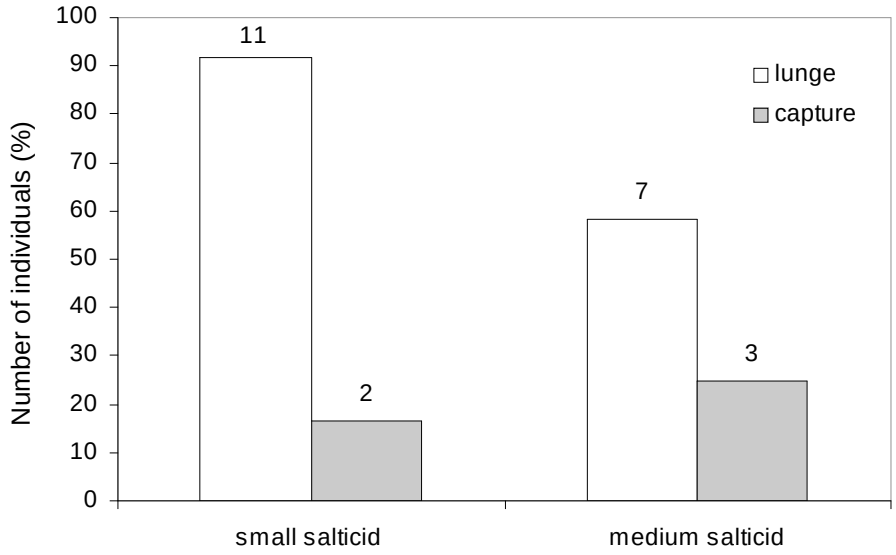

Figure 8. Percentage of *Cyrba algerina* individuals that lunged and captured small and medium size *Evarcha culicivora* under dim light (ND24=0.54 cd/m^2). Number of individuals that lunged and captured *E. culicivora* given on top of each bar (N=12).

Discussion

Salticids are renowned for their elaborate vision-mediated predatory behaviour (Forster 1982a, Harland & Jackson 2000). Yet there are reports from experimental studies of salticids capturing of prey in complete darkness (Forster 1982a,b, Taylor *et al* 1998). Caution should nevertheless be taken when interpreting the findings from these laboratory experiments. The spider's behaviour of attacking another animal may sometimes be clearly directed at the other animal as being prey, but this may not always clearly the case. Sometimes it may be more appropriate to envisage an attack on another animal, even if it is similar in size to typical prey, as a primarily defensive manoeuvre. For instances, to attack might often have a function more directly concerned with anti-predator defence rather than simply predation. Taylor *et al* (1998) acknowledged this complication and suggested that, in most instances, the observations they made of salticids encountering other arthropods in the dark were more appropriately interpreted as being primarily anti-predator defence rather than primarily prey-capture behaviour. Of course, if a salticid kills and then eats an arthropod after attacking, regardless of what the original motivation for attacking might have been, the sequence as a whole results in predation. Anti-predator defence and prey-capture behaviour may not always be clearly separate phenomena, but these considerations highlight how we really need more detailed information about what happens in the dark when salticids encounter potential prey. For example, it would be useful to know whether the salticid adopts the specific postures and behaviour that typically precede predation in the light (e.g., crouching).

Trite planiceps, a New Zealand salticid may be an especially likely candidate species for demonstrating specific adaptation to predation in the dark. *T. planiceps*' microhabitat is the dark, confined spaces inside rolled-up leaves of flax plants (*Phormium tenax*) and cabbage trees (*Cordyline* spp.) (Forster & Forster 1999), and, in contrast to most other salticids tested, this species readily captured prey in complete darkness (Forster 1982b, Taylor *et al* 1998). Perhaps *T. planiceps* has specific adaptations (e.g. reliance on chemical and vibratory cues from prey) for capturing prey in this unusual and dark microhabitat (see Taylor & Jackson 1999).

The work presented in earlier chapters suggests that *C. algerina* is also a viable candidate species for showing specific adaptation to prey-capture in complete darkness (i.e., access to olfactory cues from certain prey species influence *C. algerina's* behaviour, see Chapter 5). Yet, in the present chapter, I found no evidence to support this hypothesis. *C. algerina* did not capture any of the prey species used during the encounters staged in the dark, even when species to which *C. algerina* is known to respond to olfactorily were used.

These results suggest that, at least in the case of Sintra *C. algerina*, olfactory and vibratory cues from prey are not sufficient for prey capture to occur in the dark. Optical cues appear to be necessary for Sintra *C. algerina* to capture prey. This is in contrast to findings for the Baku *C. algerina* (Guseinov *et al* 2004), as the Baku *C. algerina* was shown to capture oecobiids in complete darkness. It is unclear why the Sintra and Baku *C. algerina* might differ in the level to which they rely on non-visual predation, and future work might consider a number of hypotheses. For instance, the higher temperatures that prevail during the summer in Baku may be a factor. In contrast to the Sintra *C. algerina*, the Baku *C. algerina* may avoid temperature in the summer by adopting a microhabitat in which ambient light is lower than in the typical microhabitat of the Sintra *C. algerina.* The summer microhabitat of the Baku *C. algerina* might be, for example, deeper under stones, or even underground. This hypothetical microhabitat difference might have been a factor that favoured the Baku population having a greater facility at predation in complete darkness. Another possibility is that differences in the behaviour of *C. algerina*'s prey in the different habitats have predisposed the Baku, but not the Sintra, population to evolve the ability to capture prey in complete darkness.

Although decreasing light levels compromised the Sintra *C. algerina*'s prey-capture success, severe effects only occurred below 0.54 cd/m^2. Below this light level there seemed to be especially adverse effects on *C. algerina*'s ability to identify and capture moving prey, prey capture occurring only rarely. Unlike when under full light, *C. algerina* no longer oriented its body immediately towards the prey and instead took an additional 1-3 s before orienting towards the prey. Accuracy of orientation also appeared to suffer, with a discrepancy of c. 10º between the prey's location and *C. algerina*'s final orientation being typical when light was below 0.54 cd/m^2. How long *C. algerina* maintained its orientation towards motionless prey also steadily decreased as light levels decreased, becoming reduced to a few seconds under the dimmest light level used (0.11 cd/m^2). Once light levels were lowered to 0.11 cd/m^2, *C. algerina* stopped tracking prey; although *C. algerina* was still capable of orienting towards the prey with reasonable accuracy, it never tracked the prey as it moved around the arena.

C. algerina seemed to remain effective at detecting prey at light levels below which it seemed to lose ability to identify prey. An explanation for this might be derived from understanding the division of labour inherent in the design of the salticid visual system. Unlike the eyes of mammals, where a single eye has an area dedicated to peripheral vision (i.e., the peripheral retina) and an area dedicated to high resolution (i.e., the fovea), salticids have gone a long way toward dividing these functions by two types of eyes, confining high resolution necessary in the identification of objects to the principal eyes, while using lower resolution

secondary eyes in the detection of peripheral movement (Land 1969a, 1981). Compared to the principal eyes, salticid secondary eyes have a much lower spatial resolution - between 0.4 and 2º compared to 0.04º in *Portia fimbriata* (Williams & McIntyre 1980, Land 1985) - but a much higher sensitivity - about ten times higher in *P. fimbriata*'s case (Warrant & McIntyre 1993).

Given the different sensitivities of salticid secondary and principal eyes, failure to maintain orientation and to track the prey after orienting may be a consequence of the principal eyes' lower sensitivity; although below 0.54 cd/m^2 *C. algerina*'s secondary eyes are still capable of detecting movement and orientation toward a moving object may still take place, the light available might be insufficient to permit prey identification by the principal eyes.

Interactions between *C. algerina* and *E. culicivora* revealed that *C. algerina* can see better than *E. culicivora* under dim light (0.54 cd/m^2); *C. algerina* often captured *E. culicivora*, but *E. culicivora* never showed any kind of reaction to *C. algerina*'s presence (i.e., *E. culicivora* never oriented, displayed or moved away), indicating that this species was probably not aware of *C. algerina*'s presence. While this may not seem so surprising, if the amount of light available in the habitat of these two species is taken into consideration an interesting question remains. How does *C. algerina* manage to see so much better than *E. culicivora* under dim light?

Animals living under dim light conditions face a common problem, how to ensure visual performance given the low number of photons available? The only way to overcome this problem is to increase photon capture (i.e., sensitivity). This can be done: 1) optically by widening the pupil, having wider photoreceptors, by having lenses with shorter focal lengths, or by having a tapetum (i.e., a light reflecting structure inside the eye that gives the retina a second chance of capturing the photons missed on the first pass) (Land 1981, Warrant 1999, Land & Nilsson 2002); and 2) neurally, by summing photons in space, (through the coupling of neighbouring visual channels - spatial summation), or in time (by extending the time (integration time) during which a sample of photons is counted by the visual system - temporal summation) (Laughlin 1990, Warrant 1999).

The price to pay for an increase in sensitivity is always a loss in resolution in space or time; when spatial summation is adopted, a loss in terms of spatial resolution occurs as the input of more and more visual channels are coupled together. If, on the other hand, temporal summation is adopted, the retina's spatial resolution remains much the same, and although the retina is able to sample a brighter image, an increasing degradation in the resolution of moving objects occurs with the use of longer integration times (Warrant 1999). Which strategy (spatial *versus* temporal summation) an animal adopts seems to be directly related to its way of life and, therefore, to its visual needs. Temporal summation is usually adopted by nocturnal sit-and-wait

predators, such as the toad *Bufo bufo* (Warrant 1999), and the net-casting spider *Dinopis subrufus* (Laughlin *et al* 1980), that need to see small moving objects. Animals that need to see fast moving objects in dim light, such as nocturnal bees (Warrant *et al* 1996, Greiner *et al* 2004a,b, Warrant *et al* 2004, Kelber *et al* 2006) and the crepuscular dung beetle *Onitis alexis* (Warrant 1999), usually adopt spatial summation, choosing temporal over spatial resolution. Although fine detail is lost with the adoption of spatial summation, enough is preserved to allow detection of coarser but rapid changes during flight (Warrant 1999).

Results from Chapter 8 indicate that, when compared to the principal eyes of more advanced salticids, *C. algerina*'s principal eyes have a somewhat shorter focal length and wider photoreceptors. Together these two factors might be responsible for *C. algerina*'s superior visual sensitivity under dim light. However, *C. algerina*'s behaviour under the dimmer light levels used suggest that *C. algerina* may also be using temporal summation to extend its visual abilities. *C. algerina*'s delayed response when orienting to prey under dim light (i.e., when light level was less than 0.54 cd/m^2) may be indicative that the spider is using longer integration times so as to increase the eye's sensitivity. If *C. algerina* is indeed using temporal summation this might also help explain the loss of accuracy when orienting towards prey; because images are sampled for longer periods of time, the resolution of moving objects is significantly degraded, small moving objects being captured as smears and, consequently, their exact location cannot be determined so precisely (Warrant 1999). Further work is, however, necessary to determine whether *C. algerina*'s visual abilities under dim light are in fact extended by temporal summation.

Although salticids are clearly adapted for a life under bright light (Land 1985, Land & Nilsson 2002), the use of optical (Chapter 8) and neuronal mechanisms may have allowed *C. algerina* to explore a niche (the undersides of stones), which due to its low ambient light levels, is probably not available to the majority of salticids. Additionally, *C. algerina*'s unusual ability to perform under dim light, may allow *C. algerina* to be especially capable of preying on more ordinary salticids (i.e., salticids such as *E. culicivora* that do not have eyes that perform well under dim light). Even though salticids do not represent a big part of the prey records in Baku (Guseinov *et al* 2004), Sintra or in the Algarve (Chapter 2), *C. ocellata* from Kenya is known to prey especially often on salticids that seek shelter under stones before nightfall (RRJ pers. com.).

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CHAPTER 10

Discussion

With more than 5000 species described, jumping spiders are by far the largest spider family (Platinick 2007). Renowned for their highly elaborate vision-mediated behaviour, most salticids actively hunt for prey in the open, making little or no use of webs during predatory events (Richman & Jackson 1992). A few salticid species from a primitive subfamily, the Spartaeinae (Wanless 1984a, Maddison & Hedin 2003), have, however, developed somewhat unusual behaviour. In contrast to most jumping spiders, the majority of the spartaeines studied to date are versatile predators (for definition see: Curio 1976), using a diverse array of specialised predatory tactics, each specific to a particular type of prey or situation. Besides being effective cursorial predators of insects, spartaeines are also known to invade alien webs and perform aggressive mimicry, making web signals on the web silk, as part of a strategy of communicating with and deceiving the web-building spiders on which they feed (Jackson & Hallas 1986a,b, Jackson & Wilcox 1993, Jackson *et al* 1998). Various spartaeines are also known to eat other spiders' eggs, and practise kleptoparisitism, entering the webs of other spiders and robbing them of their insect prey (Jackson & Blest 1982, Jackson & Hallas 1986a,b, Jackson 2002).

Numerous examples of interpopulation variation in predatory strategies have been documented among Spartaeines, especially in the genus *Portia*. Geographically separated populations of single species of *Portia* are known to adopt distinctively different innate predatory strategies, with these strategies being adaptively fine-tuned to local prey (Jackson & Hallas 1986a, Jackson & Wilcox 1990, 1993, Jackson *et al* 1997, 1998, Jackson & Carter 2001, Jackson *et al* 2002). Similarly to *P. fimbriata*, *Cyrba algerina*, another spartaeine species, is also known to be a versatile predator adopting specialised predatory behaviour against a variety of prey types (Jackson & Hallas 1986b, Jackson 1990). Although most spartaeines are tropical species found primarily in Africa and Asia, *Cyrba algerina* is distinctive, being found primarily in xeric habitats. Stretching from the Canary Islands through the Mediterranean Region and into Central Asia, *C. algerina* has the widest geographic distribution known for any spartaeine, and is the only species with a wide distribution outside the tropics (Wanless 1984a). *C. algerina*'s microhabitat is also unusual compared to that of most salticids. Although typical salticids live in brightly lit habitats and hunt their prey in the open, *C. algerina* lives on the underside of stones on the ground, a microhabitat with very low ambient light levels.

How microhabitat, together with an extensive variety of prey types over a wide geographic range has influenced the evolution of interpopulation variation in the predatory strategies of *C. algerina* has been the central question of this thesis. This thesis was divided in three parts. The first part considered the natural history (Chapter 2), the phenology (Chapter 3) and the prey-specific predatory behaviour (Chapter 4) of the Algarve and Sintra (Portugal) populations of *C. algerina*. In the second part I investigated the sensitivity of *C. algerina*'s populations to the odour of sympatric and allopatric spider and insect prey species (Chapter 5), as well as its odour-based choice between two sympatric prey species, *Oecobius machadoi* and *Trachyzelotes bardiae*, to which *C. algerina* was shown to be attracted (Chapter 6). Chapter 7 considered the influence of previous experience with prey on *C. algerina*'s prey-choice behaviour using vision- and odour-based cues. Finally the third part of the thesis considered the optics and histology of *C. algerina*'s anterior median eyes (Chapter 8), and *C. algerina*'s visual abilities under dim ambient light (Chapter 9).

PART I

Geographic variation in behaviour

Phenotypic Plasticity

As with any other phenotypic trait, behaviour is a product of both genotype and environment, and, like other phenotypic traits, often exhibits geographic variation. Variation in behaviour is sometimes a consequence of underlying genetic differentiation (i.e., genetically determined variation that occurs irrespective of particular local environmental conditions - behavioural ecotypes), but it can also be attributed to phenotypic plasticity (i.e., environmentally determined variation), or to a genotype-by-environment interaction (i.e., variation in the level of plasticity expressed by genotypes - genetic variation in plasticity) (van Noordwijk 1989, Stearns 1989, Scheiner 1998, Thompson 1999).

Phenotypic plasticity, the ability of a genotype to produce two or more phenotypes in response to environmental conditions, is central to many ideas in evolutionary biology. After a period of neglect, the study of phenotypic plasticity is becoming increasingly common, with numerous studies documenting the occurrence of phenotypic plasticity in a wide variety of species and traits. Nevertheless, a great deal of confusion in the use of terminology in this field of research still exists (Schlichting 1986, Scheiner 1993, Pigliucci 2005). A common misconception when referring to phenotypic plasticity is that plasticity is a general property of

the whole genotype. This is, however, not true. As Bradshaw (1965) and others after him (West-Eberhard 1989, Pigliucci *et al* 2006) have clearly stated, the type, the direction and degree of plasticity is specific of a particular trait, and in relation to particular environmental influences (i.e., a particular trait may be plastic in response to one environmental factor but not to another).

Another common misconception regarding phenotypic plasticity is that it represents a nongenetic means of responding to environmental changes (for a discussion see Schlichting 1986, West-Eberhard 1989). Although the exact mechanisms of evolution are still currently under debate (i.e., are there genes for plasticity, is selection acting on plasticity itself, or does phenotypic plasticity arise as a by-product of natural selection on the phenotypic values of the different character states? (see Via *et al* 2005)), it is now accepted that plastic responses to environmental variation have just as firm a genetic basis as other traits (Schlichting 1986, Scheiner & Lyman 1991); plasticity itself is a trait subject to natural selection and evolutionary change (Bradshaw 1965, West-Eberhard 1989, Scheiner & Lyman 1991, Scheiner 1998). Finally, phenotypic plasticity is not always adaptive in the evolutionary sense of increasing the animal's fitness (West-Eberhard 1989, Riechert 1999, Pigliucci *et al* 2006); some traits may be plastic because of unavoidable constraints, but to be adaptive the phenotype's response to environmental stimuli must be appropriate (e.g., although a plant may reduce leaf area under shade conditions, its fitness is not likely to be enhanced by this response) (Schlichting 1986).

Geographic Variation in Cyrba algerina

Similarly to *P. fimbriata* (Jackson 1992, Jackson *et al* 2002), *C. algerina* populations have also evolved specialised predatory tactics, which they use specifically against particular types of prey (Chapter 4), interpopulational differences in the predatory tactics and sensitivity to the odour of prey coinciding with differences in prey species availability in the habitats of each population (Sintra and the Algarve) (Chapter 2 & 5); *C. algerina* individuals from Sintra and the Algarve used a specific prey-capture behaviour when hunting bristletails. Sintra *C. algerina* used two additional distinct prey-capture tactics, one when hunting oecobiids and yet another one when hunting *Trachyzelotes bardiae*, two prey species that are common in Sintra but apparently absent in the Algarve (Chapter 2).

However, unlike the situation with *P. fimbriata*, these interpopulation differences in *C. algerina*'s behaviour do not seem to represent behavioural ecotypes. Instead, expression of *C. algerina*'s sensitivity to odour and optical cues from particular types of prey requires previous experience with the particular prey type. Furthermore, this ability is not restricted to individuals from a particular population; both Sintra and Algarve *C. algerina* develop similar odour- and

vision-based preferences for particular oecobiid species if exposed to it, regardless of whether it is a sympatric species or not.

Most surprising is the fact that the acquisition of preference for prey seems to be under the control of a developmental switch, analogous to the developmental switch that determines the sex of many reptiles species (Ciofi & Swingland, 1997), and apparently restricted to a particular prey group; encountering and preying on oecobiids appears to trigger this innate switch mechanism in both the Algarve and the Sintra *C. algerina*. Such specificity towards oecobiids is not totally unexpected; oecobiids, *O. machadoi* in particular, is one of the most common spider species in *C. algerina*'s habitat in Sintra (Chapter 2). The fact that Sintra *C. algerina* is clearly able detect this species presence using olfactory cues (Chapter 5), and has a specific predatory tactic to capture this prey (Chapter 4), suggest that *O. machadoi* is an especially important prey to Sintra *C. algerina*. These findings suggest that a similar mechanism may also exist in relation to other biologically relevant species, such as *Trachyzelotes bardiae*, a common prey species of *C. algerina* in Sintra (Chapter 2), and for which this population seems to have evolved a preference (Chapter 6), as well as specific prey-capture behaviour (Chapter 4).

In addition to interpopulation differences in behaviour, morphological and developmental differences were also encountered between the Sintra and Algarve individuals; field collected females from Sintra were considerably bigger in body size than Algarve females (Chapter 2), and this same trend was found when *C. algerina* individuals from the two populations were reared under standardised conditions in the laboratory (Chapter 3). The origin of body-size variation cannot, however, be safely determined; although spiders were reared in the laboratory under similar conditions both in terms of abiotic and biotic factors, we can not rule out the possibility that the differences observed are a consequence of maternal effects (in terms of maternal provisioning or health during pregnancy), as the spiders were offspring from field-collected females. Additional studies are needed using F2 generation from laboratory rearing.

Sintra and Algarve females were also shown to have different reproductive strategies (Chapter 3). Like other salticids (Taylor & Peck 1975, Jackson 1978, Matsumoto & Chikuni 1987), *C. algerina* is iteroparous, reproducing several times over its lifetime. Although the total number of eggs laid by Sintra and Algarve females was similar, Sintra females made a bigger investment (i.e., produced a greater number of eggs) in the first batch than in the second batch, while the Algarve females made similar investments in the two batches of eggs.

Differences were also found in the timing of a long-duration instar (Chapter 3); both populations underwent an instar of considerably longer duration, up to about three to four times longer than the other instars. In Sintra spiderlings, the long-duration instar occurred after the

spiderlings second moult (corresponding to the second instar). However, in Algarve spiderlings, the long-duration instar occurred after the spiderlings first moult (corresponding to the first instar).

Perhaps differences in the reproductive effort made by females are related to differences in the availability of prey found at each location. By increasing reproductive effort during a spring peak of prey abundance, Sintra females potentially increase the chances of a great number of its offspring surviving. In the Algarve, given the apparent low availability of prey all year round, a more modest size batch may be optimal. Differences in the timing of the long-duration instar in the two populations, could similarly be related with food availability and represent an attempt to synchronise the spiderlings development with the different environmental conditions experienced by the populations (e.g., the presence of prey of adequate size).

Phenotypic Plasticity *versus* Ecotypes

Theory predicts that phenotypic plasticity should be favoured over local adaptation (i.e., variation at the genotype level) in heterogeneous environments (Scheiner 1998, Via *et al* 1995, Foster & Endler 1999), as it is unlikely that under different environmental conditions a single phenotype confers high fitness in all situations (Via *et al* 1995). However, because behavioural divergence among populations is subject to being counterbalanced by the homogenizing influence of gene flow, behavioural ecotypes are only expected when the spatial scale of variation in natural selection is greater than the scale of gene flow (Endler 1977 *in* Thompson 1999). Only when the spatial scale of selection is smaller than the scale of gene flow, should adaptive phenotypic plasticity be expected (Bradshaw 1965).

That the spatial scale of selection is smaller than the scale of gene flow between populations seems likely for populations of *C. algerina*. Although most spiders are known to disperse hundreds of kilometres by ballooning (i.e., aerial dispersal by letting themselves be carried passively in the air on their own silken threads), sampling of ballooning spiders indicates that salticids constitute only a very small percentage (1.6–1.8%) of the spiders in aeroplankton (Greenstone *et al* 1987). Additionally, not all habitats provide similar aerodispersal possibilities, species living in less exposed habitats, such as the leaf-litter (or the undersides of stones), being poorer candidates for ballooning than those living in open areas (Patoleta & Zabka 1999). Given this, the probability that migratory events occur between Sintra and Algarve sites might be especially low.

Yet aerial dispersion might not be necessary for genetic exchange to occur between *C. algerina*'s populations. Although no formal studies of *C. algerina*'s distribution in Portugal

exist, field work during the course of this thesis revealed that *C. algerina* is commonly found all over the central and southern regions of Portugal. It now seems that instead of two isolated populations (as previously though), the Sintra and Algarve sites may in fact be part of a single much larger population, extending from the south of Portugal to at least the central part of the country. Even if no single continuous population exists, numerous smaller populations may promote some level of gene exchange between sites without the aid of aerodispersal. If such is indeed the case, then the scale of gene flow (i.e., migration) between Sintra and Algarve sites should be much larger than the scale of variation (i.e., the species of prey available to *C. algerina* in the Sintra and Algarve sites), in which case, environmental induced variation (i.e., phenotypic plasticity) should be favoured over genetically determined variation (i.e., behavioural ecotypes).

Biological Advantage of Plasticity

A significant advantage of plasticity over genetically based variation in behaviour is the greater adaptability it confers individuals living in heterogeneous and unpredictable environments (Scheiner 1993, Via *et al* 1995, Price *et al* 2003); more plastic organisms may respond to novel conditions with novel phenotypes as a consequence of genotype-environment interactions that will in turn increase the number of potential evolutionary trajectories available to the population (West-Eberhardt 1989, Wilczynski & Ryan 1999, Foster & Endler 1999).

This is clearly illustrated by *C. algerina*. Even within small patches, encounters with the same particular prey-spider species do not seem to be especially reliable; by depending on environmental cues (i.e., the presence of a particular prey species) to develop specific behavioural traits (e.g. odour sensitivity towards prey), each *C. algerina* individual can adjust its behaviour according with its own particular experience. Contrary to genetically determined behaviour, where a response to environmental alteration would require genetic change, plasticity, allows individuals to respond rapidly to changes in environmental conditions (Scheiner 1993, Via *et al* 2005). *C. algerina* individuals from each generation and from every small patch may therefore respond adaptively to the presence (or absence) of biologically relevant prey species.

Considering *C. algerina*'s wide geographic distribution, and the potential for the prey encountered by each population to vary (see Chapter 2), reliance on a more plastic phenotype, with capacity to have certain behaviours being able to be "switched on" when in the presence of particular groups of prey, is probably more advantageous than a fixed, pre-programmed behaviour as that of *P. fimbriata*'s populations.

PART II

Life on the underside of a stone

Most jumping spiders are diurnal, cursorial predators that actively capture their insect prey out in the open. *C. algerina* is most unusual in this respect, its activity apparently being restricted to the undersides of stones (Chapter 2). Undoubtedly the particular characteristics of its microhabitat have many repercussions in *C. algerina*'s behaviour. One of the most obvious is probably related with the low ambient light levels under which *C. algerina* lives. Being known for their highly elaborate vision-mediated behaviour, most salticids seem in fact to depend greatly on vision in most aspects of their lives; using optical cues alone, salticids can identify mates, rivals, predators, different types of prey and environmental features up to 40 body lengths away (Crane 1949, Forster 1979, Jackson & Blest 1982, Harland *et al* 1999, Harland & Jackson 2001, Jackson *et al* 2005). An obvious question arises: How can then *C. algerina* capture its prey under such dim light conditions?

Olfaction

The detection and identification of chemicals in the environment is a faculty all animals seem to possess (Land 1983). Spiders are no exception. Considered to be the most primitive mode of communication among arachnids, chemical communication seems to have been retained in some form in all spider families (Pollard *et al* 1987), and not even the evolution of good eyesight seems to have precluded its use by salticids. In particular, numerous studies have demonstrated that chemical cues play important roles during both intra- and interspecific interactions (Pollard *et al* 1987, Taylor 1999, Clark *et al* 1999, 2000, Jackson *et al* 2002b, Jackson *et al* 2005).

Among salticids, spartaeines in particular, seem to be especially well equipped for intraspecific chemical communication. Spartaeines are characterised, among other things, by the presence of mytiliform fields, secretory organs thought to be associated with the dispersion of pheromones (Wanless 1984b). Although there has been no work designed to clarify the precise function of these structures, behavioural experiments indicate that *C. algerina* in particular, even as a spartaeine, relies on pheromones to an unusual extent during courtship (Pollard *et al* 1987).

Kairomone use, in which a heterospecific receiver exploits the emission of a chemical compound by an emitter in its own benefit (Brown *et al* 1971, Schultz 2001), has been documented for a few salticid species (see Chapter 5). The use of kairomones by araneophagic salticids during predatory encounters seems to be highly advantageous. Besides allowing the

predator to detect the presence of unseen prey in the surroundings, kairomones from prey can potentially increase the predator's attention to optical cues from prey, while allowing it to prepare itself for the encounter (Clark *et al* 2000, Jackson *et al* 2002b).

Reliance on chemical cues from common sympatric prey species may be highly advantageous to *C. algerina.* Besides allowing *C. algerina* to locate unseen prey in the surroundings, detection of kairomones from prey provide *C. algerina* with the element of surprise, allowing it to take appropriate measures to avoid being detected by the prey, and to prepare itself for the encounter by adopting appropriate capture behaviour (Chapter 4).

An additional advantage may apply to *C. algerina*. Given the low ambient light levels of this species particular microhabitat, a strong reliance on olfactory cues from common local prey may be an especially important complement to optical cues in the detection and identification of prey.

Vision in Dim Light

Eyes have evolved into many shapes, sizes and designs (Land & Fernald 1992). Which of these a particular animal adopts is usually related with the animal's life style and related visual needs. Because eyes and brain co-evolved, it is nearly impossible in most cases to determine whether an optical innovation by a particular animal group has led to an improvement in ability to process and use the new information provided, or whether the reverse has occurred (Land 1981).

Until recently it was widely accepted that especially good vision was restricted to brightly lit habitats; vision in dim light conditions was believed to be poor, both in terms of sensitivity and resolution, and restricted to the different shades of grey. Extraordinary recent work from the vision laboratory in Lund has, however, undermined this view. Among other things, the Lund researchers have shown that the ability to distinguish colours is not restricted to diurnal animals, also occurring in some species of nocturnal moths and geckos (Kelber *et al* 2002, 2003, Roth & Kelber 2004, Kelber & Roth 2006). Some nocturnal animals can also detect movement (Warrant 1999), learn visual landmarks (Warrant *et al* 2004, Kelber *et al* 2006), use the moon's polarisation pattern and constellations of stars in the night sky to navigate (Dacke *et al* 2004), all of this under extremely dim light conditions (Warrant 2004).

C. algerina and all the above animals share a common problem: how to ensure visual performance given the low number of photons available? The only known solution to this problem is to increase the number of photons captured (i.e., to increase the sensitivity of eyes to light). Sensitivity can be improved optically: by widening the pupil, by having wider

photoreceptors, or by having lenses with shorter focal lengths (Land 1981, Warrant 1999); and neurally: by summing photons in space, or in time (Laughlin 1990, Warrant 1999).

While the principal eyes of salticids seem to have evolved towards high spatial acuity vision during daylight, the large postero-median eyes of the net-casting spider *Dinopis subrufus* have apparently evolved in the opposite direction, becoming specialised in the detection of movement in the dark (Laughlin *et al* 1980). In fact, *D. subrufus* has, what probably are, the most sensitive eyes in the spider world (Land & Nilsson 2002). The extraordinary sensitivity of *D. subrufus'* eyes is achieved through the use of both optical and neural mechanisms; besides having extraordinary big eyes with very short focal length, *D. subrufus* has wide and tightly packed photoreceptors, and is also known to make use of temporal summation to improve photon catch (Blest & Land 1977, Laughlin *et al* 1980). Although some loss in terms of resolution occurs, the retina can sample a much brighter image, allowing *D. subrufus* to capture its prey in the forest at night (Laughlin *et al* 1980).

When compared to the eyes of *D. subrufus*, or to our own eyes, the sensitivity of *C. algerina*'s eyes might seem insignificant. Nevertheless, through the use of optical and neuronal mechanisms, *C. algerina* seems to have found a way to increase its visual performance under dim light conditions, while bearing eyes known for being adapted for high acuity vision under brightly lit conditions. Yet there are some special characteristics of *C. algerina*'s eyes that, compared to more typical salticid species (i.e., species living in brightly lit habitats) confer enhanced visual performance under dim light. *C. algerina*'s principal eyes have a shorter focal length and have a smaller magnification power. *C. algerina*'s retinal ultrastructure also seems to help increase the eyes' sensitivity; *C. algerina*'s retina has a twin-rhabdomere arrangement that, although considered detrimental in terms of spatial resolution, should increase the eyes' photon catch. Finally, *C. algerina*'s behaviour under dim light (i.e., a delay in response when orienting to prey) suggests that *C. algerina* is making use of temporal summation (i.e., by summing photons captured over a period of time) to extend its visual capacity. Together all these features seem to be responsible for *C. algerina*'s superior visual sensitivity relatively to more ordinary salticids. This may in turn allow *C. algerina* to be an efficient predator in a microhabitat, which due to its low ambient light levels, is probably unavailable (in predatory terms) to most salticids. Adopting this microhabitat may have considerable advantages, including avoidance of temperature extremes, characteristic of the xeric habitats in which it lives, and that would often apply out in the open.

Future research

That I ended up with more questions than what I started with is probably not surprising. Yet the way *C. algerina* revealed itself to be substantially more complex than expected was rather extreme and the wide spectrum of unplanned topics touched on in this thesis clearly indicates that there is a strong need for further research on the biology of this species.

Additional field work will be important, especially on the Algarve population. Work should also be extended to other localities in Portugal and beyond. This holds promise of revealing especially interesting examples of intraspecific geographic variation in behaviour. Common garden experiments are needed for clarifying the relative role of genetic differentiation and phenotypic plasticity on traits that vary among populations. Besides behaviour, this should include further work on the determinants of body-size variation and different reproductive strategies.

Olfactometer work should be extended to other common sympatric prey species, as well as to adult males and the different developmental stages of *C. algerina*. The odour conditioning experiments opened a potential for an almost endless new field in salticid research. Many questions are begging for thorough investigation. For example, is a single feeding event enough to trigger the preference for a given prey? Besides oecobiids, for what other prey types might similar switch mechanisms apply? Does *C. algerina* have a more general template for a wider category of prey (e.g., *Oecobius* spp.) instead of a species-specific criterion? Do all *C. algerina* populations share species-specific switch mechanisms for the same prey species, or, on the contrary, have different populations evolved switch mechanisms for different prey types? Do *C. algerina*'s specialised predatory tactics, resemble sensitivity to prey odour by requiring previous experience with prey in order to manifest themselves?

Studies based on population genetics would no doubt answer many critical questions. For example, is there significant gene flow between the Sintra and the Algarve, or is gene exchange restricted primarily to a smaller spatial scale? How much genetic differentiation underlies the behavioural differences found between the Portuguese and the Azerbaijan populations?

However, if I had to pick one area for future research as highest priority, it would be to follow up on the initial behavioural experiments and histological work concerned with *C. algerina*'s ability to see so well under dim light. The biggest frustration from my thesis is that I did not appreciate sooner that my study animal had extraordinary ability to see under dim light. By the time I started research on *C. algerina*'s eyes, it was too late to take the eye research much beyond only scratching the surface.

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