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Evolution of prey specificity via three steps

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Abstract. Comparative experiments with two extant sister-species of *Chrysopa* provide the first demonstration of mechanisms whereby a general predator (e.g., a *C. quadripunctata*-like ancestor) may have given rise to a specialist (*C. slossonae*). First, the generalist expresses characteristics (e.g., phenotypic plasticity in larval and adult behaviour, defence-related morphology) that promote establishment on the specialist's ant-tended prey. Second, the patterns of variation among populations of the generalist suggest heritability and repeatability in traits that influence survival and reproduction on the specialist's prey – a requirement for evolutionary adaptation to the specific prey. Third, sympatric populations of the generalist and specialist have evolved reproductive isolation, thus maintaining prey fidelity. This descriptive model appears broadly applicable to predacious as well as herbacious insects.

Key words. *Chrysopa*; prey specificity; general predator; speciation; behavioural plasticity; individual repeatability; reproductive isolation; geographic variation.

Either explicitly, or more often by implication, virtually all evolutionary discussions of food associations in arthropods presuppose that the pathway to specialization involves three general steps: first, the establishment of a population of general feeders on a specific food; second, evolutionary adaptation to the specific food via natural selection on heritable traits that influence the survival and reproductive success of individuals on the new food; third, the maintenance of host fidelity through the evolution of reproductive isolation between the newly evolved specialist and its progenitor. These three steps form the essence of a descriptive model for the evolution of food specialization in animals.

Data from a prodigious number of experimental and comparative studies of insect-plant interactions indicate that this model provides a general framework for investigating how host specialization evolves in herbivores[1-8]. However, the usefulness of the model in examining the evolution of prey specialization in carnivorous arthropods remains unknown. For example, few comparative studies of phylogenetically related predacious arthropods have demonstrated mechanisms underlying predator-prey associations[9,10]; moreover, there is a paucity of data on variation in foraging, feeding, and defensive behaviour in predacious arthropods[11]. Here we provide the first report of comparative experiments using closely related predacious species to explore the evolution of predator-prey associations in insects. The findings indicate that the above three-step model is useful in explaining the evolution of prey specialization in predacious insects.

Our study focused on two predacious lacewings with traits that are highly suited for an evolutionary analysis of prey specialization. First, the two species have divergent food associations. *Chrysopa slossonae* is a specialist that feeds on the woolly alder aphid, *Prociphilus tesselatus*[12]. In contrast, *Chrysopa quadripunctata* is a generalist that feeds on a variety of soft-bodied arthropods[13]. Second, *C. quadripunctata* and *C. slossonae* are derived sister-species within a clade of general feeders[14]. This phylogeny supports the hypothesis that specialized feeding is the evolutionarily advanced state in *Chrysopa*, and it allows us to use comparisons between the two species to infer how specialization arose[15]. Third, *C. quadripunctata* (the generalist), with its diverse habitats and broad distribution throughout North America[13], expresses considerable variation within and among geographic populations. Therefore, we can combine intraspecific analyses with interspecific comparisons to derive evolutionary pathways and to test the applicability of the three-step model to predacious insects.

Step 1 – Establishment on a novel prey. C. slossonae females restrict oviposition to leaves and twigs of alder trees that are infested with woolly alder aphids[9], and their larvae develop in the large, wax-covered aphid colonies that occur on the branches and trunks of the trees. Generalist (*C. quadripunctata*) females occasionally oviposit on alder infested with woolly alder aphid colonies[9]. This behavioural flexibility in the generalist fulfills a significant requirement for the first step of the three-step model; it ensures that some generalist larvae of the *C. quadripunctata*-like ancestor occurred in the habitat where they encountered the woolly alder aphids. However, it does not ensure their access to the colony;

aggressive aphid-tending ants may form a major biological barrier between the predators and the woolly alder aphid[16]. Therefore, circumvention of ants constitutes another critical feature of the first step in the evolution of specialized feeding on the woolly alder aphid.

Specialist (*C. slossonae*) larvae avoid attack from ants through camouflage; they place copious amounts of the aphids' waxy filamentous secretions onto their dorsa where they are held by long, recurved setae and large thoracic and abdominal tubercles (fig. A). Without this defence, ants and perhaps other natural enemies remove the predacious larvae from the colony[16]. To assess whether a generalist ancestor could have avoided the ants, we compared *C. quadripunctata's* defensive behaviour and larval morphology with those of the specialist. Our comparisons showed that generalist larvae can perform the same type of camouflaging behaviour as the specialist (table 1) and that they possess similar morphological structures for holding the camouflaging material (fig. A and B).

It is especially noteworthy that like *C. slossonae*, *C. quadripunctata* larvae consistently respond to the woolly alder aphid's filamentous secretions with a high level of camouflaging behaviour; in the presence of the aphid's secretions the rate of the generalist's camouflaging activity approaches that of *C. slossonae* larvae (table 1; also see ref.[17]). Such behavioural plasticity, in conjunction with the morphological traits that subserve camouflaging, could have promoted the generalist ancestor's establishment and persistence on the ant-tended woolly alder aphid. Indeed, our recent field observations indicate that some *C. quadripunctata* larvae can complete their development in ant-tended colonies[18] – satisfying, wholly or in part, Step 1 in the evolution of prey specificity.

Step 2 – Evolutionary adaptation to the prey. The second step in the three-step model requires that natural selection act on genetic variation in traits that increase the survival and reproduction of the predator on the specific prey. Therefore, to initiate an analysis of the generalist ancestor's adaptation to the woolly alder aphid, we recently examined the extant generalist's variation in a variety of traits that we previously had shown[9,19] to adapt the specialist to the woolly alder aphid. Our new findings demonstrate that several of the generalist's traits have a pattern of phenotypic variation that parallels an inferred pattern of variation in selection pressure associated with prey. The coincidence of these patterns of variation suggests that the generalist's traits are responsive to natural selection, and it provides the framework for future studies that explore the heritable nature of the variation. Here we present two examples: one phenological, the other behavioural.

C. slossonae has a photoperiodically regulated, largely univoltine life cycle in which the active stages are contemporary with the woolly alder aphid[9]. *C. quadripunctata's* life cycle is variable – ranging from univoltine to multivoltine, and as in *C. slossonae*, the timing of development and reproduction coincides with the seasonal

Table 1. Defensive (camouflaging) behaviour in *Chrysopa* – geographic variation among populations and individual repeatability across generations

Species Population	Type of Aphid Material	Number of Camouflaging Events 1st instar early	late	3rd instar early	late
Chrysopa quadripunctata					
California	exuviae	2.50 ± 4.35a	2.50 ± 2.51a	1.50 ± 2.32a	4.40 ± 4.60a
	waxy secretions	11.10 ± 11.86a	19.10 ± 11.36bcd	12.00 ± 14.79ab	13.90 ± 5.90a
Florida	exuviae	2.40 ± 3.20a	1.70 ± 2.87a	2.60 ± 4.33a	2.50 ± 3.47a
	waxy secretions	10.30 ± 8.99a	10.90 ± 11.38abc	29.30 ± 18.66bc	33.50 ± 13.13b
New York	exuviae	3.60 ± 3.78a	4.60 ± 5.42ab	1.20 ± 1.87a	0.90 ± 1.10a
	waxy secretions	12.40 ± 12.52a	22.40 ± 15.50cd	40.90 ± 19.25cd	42.80 ± 20.14bc
Chrysopa slossonae					
New York	exuviae	6.90 ± 6.76a	16.90 ± 8.85abc	10.80 ± 5.41a	5.20 ± 5.18a
	waxy secretions	26.50 ± 16.09b	34.70 ± 21.34d	48.60 ± 19.09d	48.50 ± 13.79c

Larvae of the generalist, *Chrysopa quadripunctata* and the specialist, *C. slossonae*, perform similar movements in camouflaging themselves (grasping, lifting and placing material on the dorsum); the main interspecific differences lie in the quantitative expression of the behaviour. Geographic variation was examined with repeated-measures ANOVA followed by Tukey's HSD test; values within each column followed by the same letters are not significantly different ($p = 0.05$). Individual repeatability across generations was demonstrated with Product-moment correlations; probabilities from larvae within each population and treatment were combined (Fisher method for combining probabilities from independent tests of significance; $X^2 = 19.748$, d.f. = 11, $p = 0.05$).

There were 10 larvae per cell; larvae were first-generation offspring of field-collected females ($N = 4–7$ females/population, with larvae from each female represented in approximately equal numbers). "Naked" larvae (without any camouflaging material on their dorsa) were introduced into an arena (900 mm^2) and observed for 45 minutes with prey (*Myzus persicae*) and one of two types of camouflaging aphid material. Each larva was observed four times during its development: twice during the first instar and twice during the third instar (early = within 20 hours of hatching or moulting; late = at least 24 hours after hatching or moulting). All tests with an individual larva employed the same type of camouflaging material.

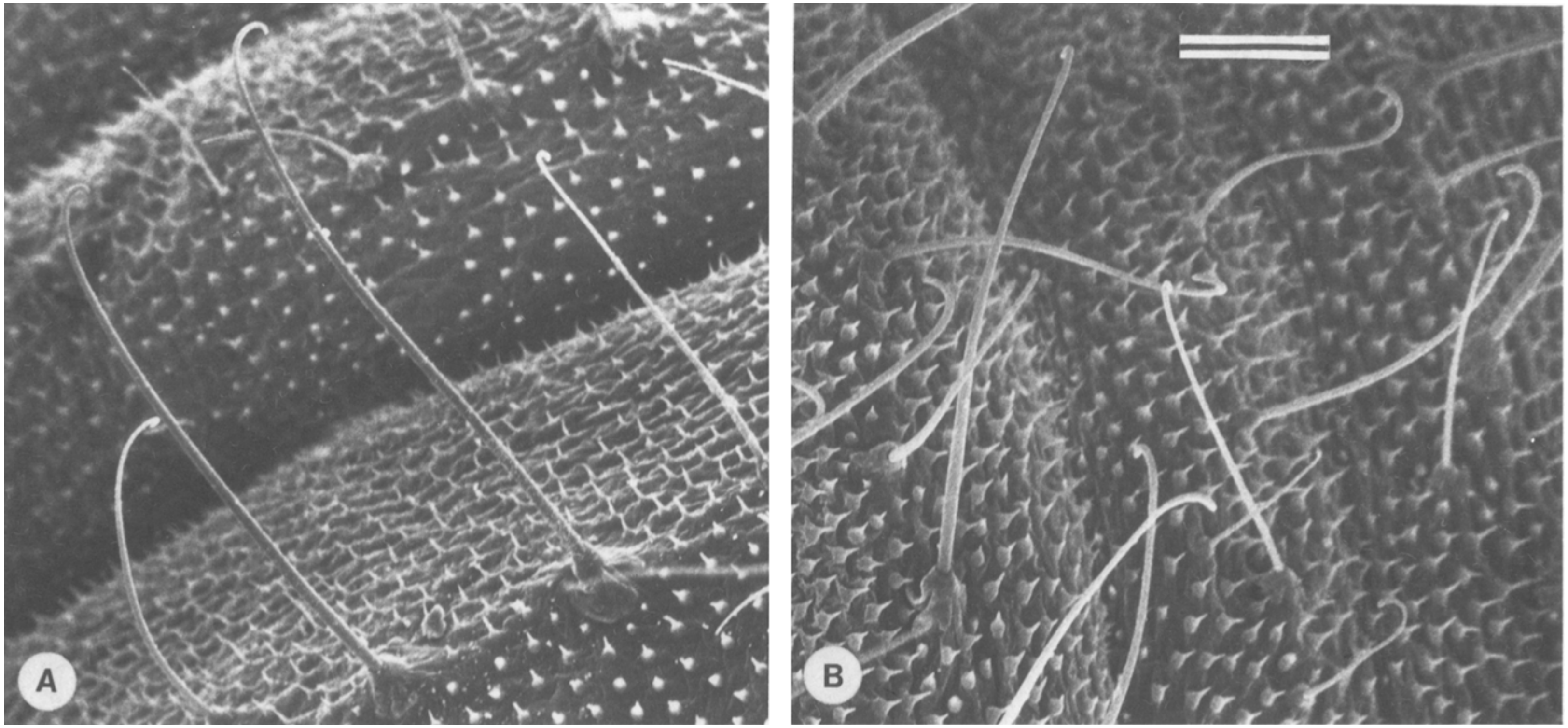

Figure. Scanning electron micrographs of the dorsal abdominal setae that hold camouflaging material on the lacewing larvae (third instars). The long recurved setae occur on both (A) the specialist, *Chrysopa slossonae*, which normally carries a large covering of waxy wool on its dorsum and (B) the generalist, *C. quadripunctata*, which is usually naked (reference bar = 50 μm).

Table 2. Geographical variation in the photoperiodic regulation of *Chrysopa quadripunctata's* seasonal cycle. The critical photoperiod is the daylength at which the photoperiodic response curve exceeds 50% diapause. Data from *C. slossonae* presented for comparison.

Population	Critical Photoperiod for Diapause Induction, Hrs. (Light: Dark)
Chrysopa quadripunctata	
Randall Co., TX	12.4:11.6
Riley Co., KS	12.5:11.5
Randolph Co., WV	13.4:10.6
Tompkins Co., NY	13.6:10.4
Yolo Co., CA	14.4:9.6
Chrysopa slossonae	
Tompkins Co., NY	15.5:8.5

First-generation offspring of field-collected females were reared under a range of seven photoperiods from L:D 10:14 to 16:8 (24 ± 1°C; $N = 21-28$ larvae/condition/population originating from 5–10 stock females/population). Offspring from each female were allocated in approximately equal numbers among all photoperiods. See reference 9 for procedures and data on *C. quadripunctata* and *C. slossonae* from Tompkins Co., NY.

availability of prey. Our recent studies showed that *C. quadripunctata's* developmental responses to photoperiod are geographically variable (table 2); furthermore, the pattern of variation tends to parallel geographic variation in the length of the prey's growing season[18]. Such geographic variability in the photoperiodic regulation of insect life cycles consistently has been shown to have a heritable basis[20], which leads us to propose that a *C. quadripunctata*-like (multivoltine) progenitor could have harboured sufficient heritable variation from which to evolve a univoltine life cycle similar to the specialist's.

Other experiments with *C. quadripunctata* lead us to suggest that the behavioural basis for *C. slossonae's* prey specificity also involved microevolutionary changes in a *C. quadripunctata*-like ancestor. First, the propensity of larvae to engage in camouflaging behaviour varies significantly among geographic populations of the generalist (table 1). Moreover, the geographic variation in this behaviour coincides with differences in larval interactions with natural enemies, notably ants[18], and we propose that an ancestor resembling *C. quadripunctata* could have carried sufficient heritable variation to evolve the specialist's complex defensive behaviour. Second, even after two moults individual larvae express significant repeatability in the level of their camouflaging behaviour. This consistency indicates that natural selection can act on behavioural variation over the entire larval stage[21]. To our knowledge this is the first experimental confirmation of individual repeatability in behaviour across larval moults for a predacious arthropod and only the second for an insect[22].

Step 3 – Evolution of reproductive isolation. The third step in the evolutionary model for food specialization – maintenance of host fidelity – involves a reduction of gene flow between the specialist and ancestral generalist. This step can occur either through direct selection on reproductive traits (in sympatry) or as an indirect consequence of differential adaptation to disparate food or other factors (in sympatry or allopatry). To differentiate between these possibilities we reasoned that if the specialist had become reproductively isolated solely as a result of adaptation to its specific food, specialist males and females in the laboratory would be no more or less likely to interbreed with generalists from sympatric populations than with those from allopatric populations. Our recent studies show that for *C. quadripunctata* and *C. slossonae*, the above expectation is not fulfilled. Interspecific pairs varied geographically both in the degree of fertility and in the symmetry of variation between the sexes. In the pairs of *C. quadripunctata and C. slossonae* from New York (pairings that involved males and females from sympatric populations), both reciprocal crosses had significantly reduced levels of fertility [table 3; *C. quadripunctata* ♀ × *C. slossonae* ♂: $G = 19.83$, d.f. = 1; $p = 0.001$; *C. slossonae* ♀ × *C. quadripunctata* ♂: $G = 4.65$, d.f. = 1; $p = 0.05$; G test of independence (Model II) with Yates correction on paired comparisons]. In the crosses involving the *C. quadripunctata* population from Florida and *C. slossonae* from New York, only pairs with *C. quadripunctata* females had significantly reduced fertility, but not the reciprocal cross (table 3; $G = 15.10$, d.f. = 1; $p = 0.001$ and $G = 0.96$, d.f. = 1; $p > 0.1$, respectively). Finally, crosses between the California population of *C. quadripunctata* and *C. slossonae* showed no significant reduction in

Table 3. Geographic variation in the reproductive isolation between *Chrysopa quadripunctata* and *C. slossonae.*

Cross	% Pairs Producing Fertile Eggs (N)
Chrysopa quadripunctata (New York)	
♀ × *Chrysopa slossonae* ♂	10 (10)
♂ × *Chrysopa slossonae* ♀	60 (10)
♀ × *C. quadripunctata* (NY) ♂ (Control)	100 (15)
Chrysopa quadripunctata (Florida)	
♀ × *Chrysopa slossonae* ♂	10 (10)
♂ × *Chrysopa slossonae* ♀	90 (10)
♀ × *C. quadripunctata* (FL) ♂ (Control)	100 (10)
Chrysopa quadripunctata (California)	
♀ × *Chrysopa slossonae* ♂	80 (10)
♂ × *Chrysopa slossonae* ♀	80 (10)
♀ × *C. quadripunctata* (CA) ♂ (Control)	100 (15)
Chrysopa slossonae (New York)	
♀ × *C. slossonae* (NY) ♂ (Control)	100 (10)

The two species occur sympatrically in eastern United States, but *C. slossonae* is absent from western U.S. (*C. quadripunctata* localities: New York = Tompkins Co.; Florida = Jefferson Co.; California = Yolo Co; all *C. slossonae* were from Tompkins Co., New York). Experimental animals were first-generation offspring of field-collected females ($N = 3-6$ stock females/population, with offspring from each female allocated in approximately equal numbers among all crosses). Infertility was distributed over the offspring of all parental females. .

fertility ($G = 0.58$; d.f. = 1; $p > 0.1$). Such a pattern of geographic variation in hybridizability supports the hypothesis that reproductive isolation did not evolve solely as a secondary consequence of divergent adaptation to different prey, and we suggest that natural selection had a direct role in the evolution of prey fidelity – either through the evolution of pre- or post-mating barriers or through character displacement, with pleiotropic effects on hybridizability.

Conclusion

Our study with two sister-species of lacewings provides experimental evidence for the applicability of a general three-step evolutionary model to predacious insects. Although studies aimed at elucidating the mechanisms involved in predator-prey associations[23] are relatively few, they are beginning to yield comparative and genetic data that appear consistent with the three-step model. For example, some predacious insects and spiders exhibit plasticity and trade-offs in predatory behaviour that allow them to utilize novel prey[24]; also some have been shown to harbour genetically based variation in feeding and defensive behaviour that can subserve adaptation to diverse prey resources[11]. From our results with the *Chrysopa* system, we conclude that comparative experimental analyses of predator-prey associations in phylogenetically related arthropods offer an array of attractive opportunities for basic and applied studies[15,25]. Not only can such studies help elucidate the mechanisms underlying the diversification and speciation of predators[26], but they can also help explain patterns in predator distribution and abundance[27]. Ultimately, they may improve substantially the ability of applied ecologists to predict the reliability and safety of using predacious insects for the biological control of arthropod pests[28].

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