

Diffuse coevolution: constraints on a generalist parasite favor use of a dead-end host

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Forbes, M. R., Muma, K. E. and Smith, B. P. 2002. Diffuse coevolution: constraints on a generalist parasite favor use of a dead-end host. – *Ecography* 25: 345–351.

Many evolutionary models and empirical studies of parasite-host interactions consider single species of parasites exploiting single host species. However, many parasites are generalists in that they parasitize more than one host species (often many more) and establish associations with other hosts that cannot be described as true parasitism. We identify such an association, explain how constraints may maintain it, and indicate why such diffuse interactions are deserving of attention. We describe the use of two closely related *Sympetrum* dragonfly species by larvae of the water mite *Arrenurus planus* Marshall. Adults of one dragonfly species are resistant whereas adults of the other species are almost wholly susceptible to *A. planus*. However, *A. planus* attaches as often to the resistant host as it does to the susceptible host species when relative abundance and seasonal timing of adult emergence of both species is considered. We present evidence that mites track the susceptible host and are most active early in the season, when early-emerging unsuitable hosts are also present. Thus, use of resistant hosts appears an unavoidable outcome of constraints promoting discovery and use of susceptible hosts. Such findings have implications for studies of local adaptation and host switching.

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Many studies on parasite-host interactions consider single parasite species exploiting single host species, usually from a narrow geographic range. Recently, this approach has been expanded to studies on local adaptation comparing parasite fitness in allopatric and sympatric races of hosts (e.g., Ebert 1994, Imhoof and Schmid-Hempel 1998). Furthermore, biologists recently have considered the fitness or success of parasites exploiting different host species (e.g., Schmid-Hempel and Schmid-Hempel 1996, Tripet and Richner 1997) or the ability of different host species to harbor more parasite species (Durrer and Schmid-Hempel 1995). These types of studies are complementary and important for understanding the co-evolutionary potential of parasites and their hosts. It is especially important because many parasite species have broad host (and geographic)

ranges and often exploit more than one species during particular stages of their life cycles (Strand and Obrycki 1996, Poulin 1998).

In some extreme cases, the parasite can enter into an association with the host that is not easily defined as parasitism. The “parasite” may encounter resistance from some host species resulting in its death (Forbes et al. 1999). The host also may not benefit because of the following: 1) it loses resources to the developing, but ill-fated, parasite, and/or 2) it pays a cost of resistance. In fact, costly resistance has been documented or strongly inferred from selection experiments and other studies (e.g., with insects subjected to natural or simulated parasitism: König and Schmid-Hempel 1995, Kraaijeveld and Godfray 1997, Fellowes et al. 1998, Siva-Jothy et al. 1998).

Accepted 1 October 2001

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ISSN 0906-7590

Maintaining resistance should be a viable option so long as resistance is less costly than actual parasitism. Occurrence of such associations might thus depend on constraints operating on parasites, although it would seem useful to evolve mechanisms to avoid, or evade defenses, of unsuitable hosts. In a previous study (Forbes et al. 1999), we reported that larval *Arrenurus planus* (Marshall) attached to larvae and parasitized adults of two related species of dragonflies: *Sympetrum internum* Montgomery and *Symptetrum obtrusum* (Hagen). The mites engorged successfully on adult *S. obtrusum*, but almost all mites died on adult *S. internum* after encountering cell-mediated resistance resulting in collapse of their feeding tubes.

Herein, we explore whether constraints foster inappropriate host choice and thus whether this association highlights loss of parasite coevolutionary potential. First, we addressed whether rates of attachment were lower on *S. internum*, which would indicate reduced use of a resistant host species. As shown below, we found that attachment rates were similar for both species, after controlling for relative host abundance and timing of host emergence. Furthermore, we suggest that tracking of the susceptible host (*S. obtrusum*), combined with constraints in exploiting other partially susceptible species, can explain persistent and inappropriate host choice by *A. planus*. Such inappropriate host choice represents an extreme case, because mites on *S. internum* die. Among-host variation in parasite fitness may be widespread in nature and has implications for testing some central co-evolutionary models.

Materials and methods

Study site and natural history of these mite-dragonfly associations

Our study site was Yezerinac's Pond (44°32'13"N, 76°22'55"W, see also Forbes et al. 1999 for a description), which supports *A. planus* mites (Acari: Arrenuridae). Based on allozyme data, *A. planus* mites show genetic differentiation across regions consistent with limited gene flow among populations in widespread ephemeral ponds (Bohonak 1999), suggesting the potential for local adaptation to host species. However, these mites are generalists in that they parasitize adult *Sympetrum* dragonflies (Libellulidae) and adults of various damselflies (Lestidae), although to a lesser extent on damselflies at our study site.

Larval *A. planus* first colonize, and are phoretic on odonate larvae, similar to other arrenurid parasites of odonates (Smith 1988, Leung et al. 2001). Once larval hosts emerge and start to eclose, mites crawl onto the newly formed imago. Once on the new adult, mites

pierce the host's cuticle using their chelicerae and secrete a stylostome or feeding tube and become parasitic (Smith 1988). Like all other "higher" water mites (members of the superfamilies Arrenuroidea, Hygrobatoidae, Lebertioidea), these mites start to parasitize at host emergence, and thus engorgement is synchronous on an individual host (Smith 1999). In other odonate-mite associations, hosts neutralize one or more stylostome(s) through melanotic encapsulation (Åbro 1979, 1982, but see Forbes et al. 1999). If resistance is not mounted, the fully engorged mite drops off when the adult host returns to the pond for reproduction (e.g., Rolff and Martens 1997). Detachment of *A. planus* from individual hosts is quite synchronous (Forbes et al. 1999). For example, few host individuals are found with both mites and mite scars, indicating that most mites probably detach during single bouts of reproduction. In another study on a dragonfly species, detachment of unidentified *Arrenurus* spp. on an individual host could occur over several days. However, more than one *Arrenurus* species was likely involved (Smith and Cook 1991). The natural history of this particular association makes it well suited to scoring mites as either alive or dead (see Forbes et al. 1999 for scoring mites as dead).

Collections, aging hosts, scoring numbers and engorgement of mites

We sampled from 7 June to 9 August, 1999. For each dragonfly netted, we counted mites on the thorax and abdomen using a 10 × or 20 × loupe. We identified host species using facial coloration, male secondary genitalia, and female vulvar laminae and aged them as teneral or mature adults using Walker and Corbet's (1975) criteria. We were confident that teneral emerged from our study pond, within 2 d of sampling. They were invariably weak flyers whose wings and bodies had not yet dried and hardened. Moreover, in both host species, the mites on teneral dragonflies were unengorged which is typical for mites that have just made the transition from host larva to recently-eclosed adult (e.g., Mitchell 1968). Mature adults may have emigrated from similar habitats.

We used 314 newly-emerged dragonflies to compare number of mites on each species. We also captured 206 mature males and females to score engorgement of mites on a scale of 0–5. We scored mites as 0 if their body was flat, with no separation between ventral sclerites and their dorsal plate and as 1 if there was a slight separation, which represented engorgement from 0.15 to 0.22 mm (n = 7). We scored mites as 2–5 if they were (respectively) 2–5 × the length of their dorsal plates. Fully engorged mites were 0.88–0.91 mm (n = 7) (cf. Forbes et al. 1999).

Results

Engorgement success of *A. planus* on the two species

We start with comparisons of fitness of parasites on mature adults of the two host species, since this is central to our later arguments. Our results confirmed that *S. obtrusum* was almost entirely susceptible to *A. planus*. Only 6 of 95 mature males had all their mites showing little or no engorgement (scores 0–2); the rest had all their mites engorging or fully engorged (scores 3–5). Similarly, 6 of 65 mature females had mites that scored 0–2; the rest had engorging or fully engorged mites (scores 3–5). In stark contrast, we found that the 43 mature male and 3 mature female *S. internum* all had non-engorging mites scored as 0 that were dead. Dead *A. planus* lose their color on *S. internum*, appearing silver instead of bright red. The difference in proportion of individuals between species showing no or little mite engorgement was highly significant (100% for *S. internum* versus 7.5% for *S. obtrusum*, $\chi^2 = 151.1$, $p < 0.001$).

Evidence for reduced use of a resistant host?

Together with a previous study (Forbes et al. 1999), these results show that *A. planus* attaches to both a resistant and a susceptible species. We did find higher mean and median abundance of mites, but not a higher prevalence, on *S. obtrusum* than *S. internum* after including all newly-emerged dragonflies (Table 1). One problem with this type of statistical description is that small samples drawn from a negative binomial distribution (typically the best description of the pattern of water mites among hosts, Smith 1988) will have comparable measures of prevalence to larger samples, but lower measures of abundance (Poulin 1998). Furthermore, mite abundance was not normally distributed for any gender-by-species category (Shapiro-Wilk's W ranged from 0.59 to 0.66, all $p < 0.0001$) thereby pre-

cluding parametric tests on non-transformed mite counts. We found that although mite numbers were normally distributed following log ($x + 1$) transformation for 3 of 4 gender-by-species combinations (W ranged from 0.94 to 0.98, p values from 0.06 to 0.92), transformed mite numbers were not normally distributed for *S. obtrusum* females ($W = 0.94$, $p < 0.001$). This group was the best-represented in samples.

Thus, we performed randomization tests to test whether there were gender differences in average mite numbers for either species. If there were no gender differences, the procedure was repeated with pooled data to determine whether there were species differences. We found that the difference in mean numbers of mites between male and female *S. internum* (22.0 mites, calculated from Table 1) was not significantly different from chance expectations (1000 repeats, $p = 0.13$), nor was the difference between male and female *S. obtrusum* (12.4 mites, 1000 repeats, $p = 0.14$). However, the difference in mean numbers of mites between *S. internum* and *S. obtrusum* (27.5 mites, males and females combined) was significantly different from chance expectations, when all individuals were considered (1000 repeats, $p < 0.001$).

Host species can, however, differ in their emergence periods and *Arrenurus* spp. mite numbers are known to change seasonally in other associations (Smith and McIver 1984, Forbes and Baker 1991). To test for species differences controlling for time of season, we first grouped the combined emergence period (time of season over which teneral were netted) of both host species into 5-d intervals and tabulated the cumulative percentage of mites found on hosts. Mite abundance started off low, but that period was of short duration and followed by a rapid increase in numbers of mites on hosts (Fig. 1). We found *S. internum* emerged earlier than *S. obtrusum*, after first showing no gender differences in median emergence dates within species, using Wilcoxon tests (Table 2). The former species tended to emerge at 4–5 d when mite numbers were low, had a less protracted emergence period (Table 2), and showed a nearly significant positive association between date of

Table 1. Numbers of male and female teneral of both host species (N) and associated statistical measures of *A. planus* prevalence and abundance for each category of host (following Bush et al. 1997). Included are the following: prevalence (and confidence limits on proportions following Zar 1996), mean abundance (and standard deviation, SD), median abundance with inter-quartile range (IQR) and full ranges (FR) observed, the sum total of mites (Sum) and the coefficient of variation in mite abundance (CV).

	<i>S. internum</i>		<i>S. obtrusum</i>	
	Males	Females	Males	Females
N	24	29	71	190
Prevalence	87.5	96.5	94.4	95.3
Confidence limits	67.5–97.3	82.1–99.9	86.1–98.4	91.1–97.8
Mean (SD)	17.5 (26.9)	39.5 (59.5)	48.0 (64.3)	60.4 (73.6)
Median (IQR)	10 (2.5–19.5)	14 (5.5–45.5)	25 (8–62)	28.5 (9.75–81.5)
FR	0–106	0–269	0–344	0–382
Sum	421	1144	3411	11 468
CV	153.3	105.7	134.0	122.0

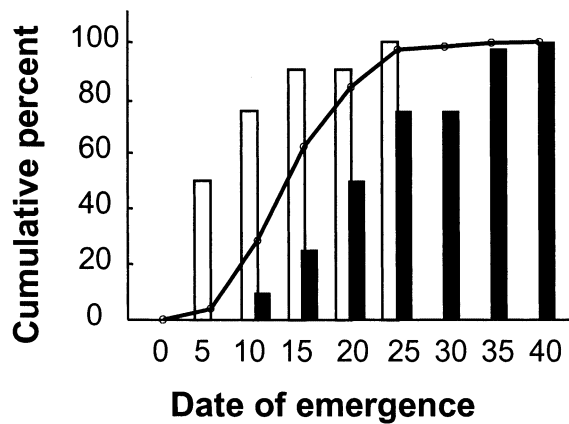


Fig. 1. Cumulative percentage of mites (based on 16 444 mites) over eight 5-d intervals for the combined emergence period of *S. obtrusum* and *S. internum* (line). Cumulative percentage of emergence for *S. internum* (open bars) and *S. obtrusum* (filled bars) for that same time period.

emergence and mite abundance (Spearman_s = 0.25, 0.1 > p > 0.05, n = 53). In comparison, *S. obtrusum* tended to emerge at 17–18 d when mite numbers were high but starting to decline (as seen from the decelerating cumulative abundance, Fig. 1) and showed a significant and inverse relation between emergence date and mite numbers ($r_s = -0.24$, $p < 0.001$, $n = 261$).

For the test of species differences in attachment of mites, we had to control for timing of emergence. Thus, we compared teneral captured on days 5–25 of the emergence period, ignoring *S. internum* captured earlier (when *S. obtrusum* were not emerging) and *S. obtrusum* captured later (when *S. internum* were not emerging). Thus, we compared only individuals of both species that emerged at the same or similar time of season. We again used randomization tests to determine whether absolute differences in mean numbers of mites, between males and females or between species, were larger than expected by chance. The observed difference of 9.2 mites between 55 male and 168 female *S. obtrusum* was as large as chance expectations (1000 repeats, $p = 0.21$).

Table 2. Tests for differences in emergence dates between males and females of *S. internum*, between males and females of *S. obtrusum*, and between both species, after males and females were combined. Included in the table is the total number of individuals for each category of host (N). Also included are the median emergence dates (day 1 = 7 June, 1999), the interquartile range and full range of dates (IQR and FR, respectively) over which each category of host emerged, and the Wilcoxon test statistic and associated p value.

	N	Median	IQR	FR	Wilcoxon Z (p)
<i>S. internum</i>					
males	24	4	3–7	1–17	–1.09 (0.27)
females	29	5	4–8.5	2–25	
<i>S. obtrusum</i>					
males	71	18	11–25	7–39	1.57 (0.11)
females	190	17	11–21	5–39	
Combined males and females					
<i>S. internum</i>	53	5	3–7	1–25	–9.70 (0.001)
<i>S. obtrusum</i>	261	17	11–24	5–39	

Table 3. Numbers of *S. obtrusum* hosts that emerged over each of seven 5-d intervals. Also shown are the mean numbers of mites and standard deviations (SD) in mite abundance for each group of hosts. See text for results based on these data.

Days	N	Mean	numbers (SD)
5–9	29	53.5	(61.0)
10–14	89	80.2	(92.2)
15–19	43	71.1	(72.0)
20–24	48	50.9	(42.0)
25–30	24	17.5	(18.7)
30–34	22	10.0	(13.6)
35–40	6	8.3	(3.8)

We also found no significant differences in mean numbers of mites between male and female *S. internum* (observed difference = of 49.8 mites; 7 males and 14 females, 1000 repeats, $p = 0.16$).

When males and females were combined for analysis, the observed mean abundance for *S. obtrusum* (64.7 mites) and for *S. internum* (45.8 mites) were not statistically distinguishable (1000 repeats, $p = 0.13$). Thus, when temporal effects were removed and representation of host species considered, *A. planus* larva showed similar attachment to both species, despite the fact that over the whole of emergence, *S. obtrusum* is the most common host. Notwithstanding, there is clearly no evidence for reduced use of a resistant species. Stated another way, the 53 *S. internum* represented 16.8% of teneral dragonflies sampled and had > 10% of mites (Table 1): those mites failed to engorge and died.

Does *A. planus* track its susceptible host?

Arrenurus planus may be constrained in tracking the common susceptible host, *S. obtrusum*. In this scenario, female mites time egg laying so that larvae hatch when they are most likely to encounter final-instar *S. obtrusum* hosts. To test this possibility, we compared numbers of *S. obtrusum* hosts emerging over 5-d intervals with mean numbers of mites carried by those hosts. The majority of *S. obtrusum* (ca 34%) emerged over

days 10–14 and carried the heaviest mite loads (Table 3). The correlation between log (numbers of hosts) emerging over seven 5-d intervals and mean numbers of mites over each of those intervals was significant (Pearson $r = 0.84$, $F_{1,7} = 11.6$; $p < 0.020$, Table 3).

Here again, the problem of small samples needs to be addressed. From days 36–40, only 6 dragonflies were sampled and had a mean abundance of 8.3 mites (Table 3). Such an effect could be due to chance. However, when this sample was removed from the analysis, the correlation between log (numbers of hosts) emerging over six 5-d intervals and mean abundance of mites was still significant ($r = 0.87$, $F_{1,6} = 12.1$; $p < 0.025$, Table 3). The next lowest sample sizes ranged from 20 to 30 hosts sampled over each of three 5-d periods (Table 3). Although comparable with respect to sample sizes, these samples varied in average mite numbers from 10 to 53 mites with larger mite numbers being present earlier in the season (Table 3). Thus, low average abundance was not simply an artifact of some small samples, nor was the relation between host availability and mite abundance. Rather, it seems *A. planus* abundance relates to availability of its common and susceptible host, *S. obtrusum*.

Discussion

Generalist parasites may provide an interesting challenge to theories on host-parasite co-evolution. In particular, generalists well adapted to certain host species may enter into interactions with other species, where successful exploitation is constrained. But apart from work on parasitoids (reviewed by Hawkins 1994), not many studies have considered fully the constraints on host use and exploitation. In this study, we demonstrated that one species was resistant to a generalist parasite whereas another species was susceptible. Yet mites use both hosts proportionately based on their representation during co-emergence. Furthermore, mites on the resistant host do not continue their life cycle. These two results suggest that constraints may be operating.

Our study indicates that both *S. internum* and parasite individuals inhibit one another. Hosts should retain costly resistance so long as it is less costly than actual parasitism. For insects including odonates, infections by water mites have been associated with fitness costs. These include reported reductions in flight ability, fecundity, mating success and/or survivorship, in both observational and experimental studies (e.g., Lanciani 1983, Smith 1988, Forbes 1991, Forbes and Baker 1991, Reinhardt 1996, Leung and Forbes 1997, Léonard et al. 1999, but see Andrés and Cordero 1998, Rolff et al. 2000). Thus, selection to maintain resistance should be present in many such hosts and is certainly seen in *S.*

internum and *Lestes* species exploited by *A. planus* (Forbes et al. 1999, Yourth et al. 2001).

What are the constraints operating on mites and might these be representative of other associations between generalist parasites and their hosts? First, host discovery is a major limiting step in the life cycle of many water mites. For example, Collins (1975) estimated that ca 90% of larval *Partnuniella thermalis* Viets do not find a host. Cook et al. (1989) also discuss the inherent challenge that a larval mite < 250 microns must have to locate dragonfly hosts that typically occur in densities < 10 m⁻². Selection against use of inappropriate hosts seems weak for *A. planus* given that > 10% of mites colonize *S. internum* and die. Furthermore, it appears that *A. planus* cannot discriminate between *S. internum* and *S. obtrusum*, given that rates of infestation are not significantly different between host species once timing of emergence is controlled. Perhaps it is very difficult for the mite to have a suitable cue to discriminate, whilst still accepting other dragonfly and damselfly species.

Female mites presumably lay eggs at a time of season when their offspring have the best chances of survival. Most larval mites are present when *S. obtrusum* numbers are quite high, meaning that the likelihood of finding an appropriate host is high, relatively speaking. This time is earlier, rather than later in the season. It is quite likely, therefore, that by laying eggs early in the season, *A. planus* larvae are more likely to find *S. obtrusum* hosts, but run the risk of also encountering early-emerging *S. internum* hosts. Later in the season, this risk of use of inappropriate *Sympetrum* spp. hosts is reduced, but so also is the likelihood of finding an appropriate *S. obtrusum* host.

Selection need not be mediated by single factors. Another study from our lab (Yourth et al. 2001) has indicated a potential cost of *A. planus* larvae being active later in the season. This species also exploits lepid damselflies. One species (*Lestes dryas* Kirby) emerges earliest in the season at our study site, and is entirely susceptible to *A. planus* mites. However, later-emerging species show variable resistance, and in the most commonly exploited *Lestes* species, the likelihood of melanotic encapsulation of mite feeding tubes is higher later in the season. Thus, several selection pressures may operate simultaneously and favor early activity of *A. planus* larvae. In conclusion, *A. planus* suffers from use of inappropriate hosts by the very mechanisms that promote use of suitable hosts: broadcast infective larvae that are active early in the season.

This type of interaction where both host and parasite individuals inhibit one another may be widespread. It is premature to suggest that these interactions may transcend into population-level phenomena, but it is intriguing that use of inappropriate hosts is persistent. Other parasites show instances where they associate with, but are unable to effectively exploit species in a

suite of potential hosts (see recent work on the generalist brood parasite, the brown-headed cowbird: e.g., Hosoi and Rothstein 2000). Even hosts not successfully exploited by parasites pay a cost (obviously, it would be better if the parasites were not challenging them). In the case of cowbird hosts, costs could include nest rebuilding or abandonment as well as aggressive responses to brood parasites. For mite hosts, resistance is likely to be costly (see Introduction). That many parasites are generalists is important, but how their success is constrained on different hosts remains largely untested. This information should help us predict the occurrence and persistence of such interactions and their implications for testing general ideas about parasite-host co-evolution.

Acknowledgements – This research was supported by a grant from Natural Sciences and Engineering Research Council, Canada to MRF and by a Provost's summer research grant from Ithaca College to KEM. The authors are indebted to the Queen's Univ. Biological Station for access to the Hilda and John Pangman Conservation Reserve and for logistical support.

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