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Influence of gut parasites on growth performance in the water strider *Gerris buenoi* (Hemiptera: Gerridae)

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Water striders harbor a diverse assemblage of symbionts in their digestive tract. We used a field experiment, in which water striders were reared in enclosures in their natural pond habitat, to assess the effects of gut symbionts on growth. Trypanosomatid flagellates had significant adverse effects on both development time and adult size, and therefore are clearly parasitic. Yet because of their low prevalences (2% or less), trypanosomatids cannot be a major factor in the dynamics of our study population. Gregarines occurred in 36% of the water striders, often in high numbers, and filling the entire midgut of some bugs. Nevertheless, infected and uninfected gerrids did not differ in their growth, and gregarine loads were uncorrelated with development time and adult size attained. We also did not find effects of gregarines in a second experiment with different rearing conditions, including a treatment with food stress. We used a quantitative genetic approach to test if resistance against gregarines has a heritable component. There was no evidence for any genetic variation, suggesting that variability in gregarine loads is the result of environmental heterogeneity. Comparison with published reports from water striders shows that there is a great variability in the diversity and prevalence of symbionts among different populations and species of gerrids.

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Diseases and parasites of insects have been investigated thoroughly for their potential in biological control of pests (e.g., Tanada and Kaya 1993). Therefore, most studies have dealt with pathogens of high virulence, whereas chronic, sublethal infections have received less attention. More recently, however, these milder forms of infection have been recognized as important factors in a variety of ecological and evolutionary contexts, and their effects on host insects have attracted more attention from entomologists (e.g., Zuk 1987, 1988, Arnqvist and Mäki 1990, Simmons 1990, Shykoff and Schmid-Hempel 1991, Leong et al. 1992, Schaub 1992, Siegel et al. 1992). Still, the interplay of genetic and environmental factors that influences the patterns of infection in natural populations is mostly unknown, and it is therefore extremely difficult to assess the impact of symbionts on the dynamics of their host populations.

Among the protozoan parasites of water striders (Hemiptera: Gerridae), trypanosomatid flagellates (Zoomastigina: Trypanosomatidae) are relatively well known because they have been studied in a variety of locations since early this century (e.g., Patton 1908, Porter 1909, Becker 1923; summary in Wallace 1966). Ecological studies examined seasonal changes in prevalences, mode of transmission, and pathogenicity of trypanosomatids in gerrids (Tieszen and Molyneux 1989, Arnqvist and Mäki 1990). Our study population of *Gerris buenoi* Kirkaldy differs from previous studies because of its much lower prevalence of trypanoso-

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matids (except for one population sampled by Becker 1923), and because of the high prevalence of other gut symbionts, most notably gregarines (Apicomplexa: Gregarinia) and ciliates (Ciliophora). These symbionts have not been reported in previous studies (except for a vague record in Poisson 1957: 16), and their effects on the gerrid hosts are unknown.

In the only study of the effects of gut parasites on gerrids, Arnqvist and Mäki (1990) found that trypanosomatids reduced the vigor of adult bugs, but did not consider their effects on larvae. Negative effects on larval growth of their insect hosts have been shown for various symbionts (e.g., Schaub 1992, Siegel et al. 1992). In gerrids, such effects are of special interest because they may explain patterns of covariation among life history traits that contradict the assumptions of currently accepted theory. Laboratory and field studies have shown that development time and adult size are negatively, not positively, correlated (Blanckenhorn and Fairbairn 1995, Klingenberg and Spence in press); estimates of genetic correlations between these two life history traits in our study population are also negative (Klingenberg and Spence in press). Effects of gut symbionts would reconcile these experimental observations with conventional models if infections by protozoan parasites account for variation in life history traits. Specifically, this explanation requires that infections common in this population prolong development time and simultaneously reduce adult size, and that the susceptibility of gerrids to these infections has a genetic component sufficient to account for the genetic variation found in life history traits.

In this paper, we present the results of rearing experiments with the water strider *Gerris buenoi* under field conditions. We determined development time, adult size, and gut symbionts for individually reared water striders, and estimated the effects of different symbionts from comparisons of infected and uninfected water striders, and from correlations of life history traits with symbiont loads (for gregarines only). We used a quantitative genetic approach to test for a genetic component of susceptibility, whereas a separate experiment with a series of different rearing regimes allowed us to assess if symbiont effects on gerrids are mediated by environmental conditions.

Materials and methods

Field experiments

We conducted our experiments from April to September 1994 on Meadow Pond, a man-made pond at the George Lake Field Site near Edmonton, central Alberta, Canada. We reared water striders individually in bottomless plastic containers (diameter 10 cm, rim ca 6 cm above water) kept afloat on the water by a ring of plastic foam glued around its outside. To protect the larvae from predators, these containers were kept in enclosures ($2 \times 1 \times 1$ m), each with four separate compartments, which were covered with screen on all sides as well as at the top and bottom (for details, see Klingenberg and Spence 1996).

The first experiment, in the spring generation, consisted of individual rearings of water striders which were the offspring of overwintered bugs collected from the study population immediately after snowmelt, but before the onset of mating. These adults were set up in a half-sib breeding design (Falconer 1989); each male was mated to three females. Eggs collected from the females were kept in the laboratory, and the first-instar larvae were transferred to the eight field enclosures within 24 h of hatching. We allocated a position in one of the enclosures to each larva with a randomized list. Larvae were checked for molts daily and fed with frozen insects (mostly chironomid midges) caught in a light trap run nearby at the field site; the amount of food given daily to each gerrid was roughly equal to its body volume, and therefore constituted an ad libitum regime. Within 24 h of the final molt, we collected the emerging adults, measured their total body length to the nearest 0.1 mm, and dissected them to identify their protozoan gut symbionts. These dissections were carried out for 506 gerrids. We calculated development times in degree-days from air temperature data taken at the surface of a nearby pond, using instar-specific temperature thresholds (Klingenberg and Spence in press).

We carried out a second experiment to establish the role of diet, interactions among larvae, and habitat structure on gut symbionts and growth performance. This experiment used water striders of the summer generation, which were the offspring of direct-breeding gerrids hatched earlier in the season. In addition to the standard rearing protocol described above, which served as a control treatment 1), we included three other treatments: 2) a treatment with reduced food levels ("food limitation"), in which the gerrids were fed every other day only, 3) a treatment that allowed direct interactions and competition among larvae ("triplet"), in which there were three larvae per container instead of one, and 4) a treatment with additional habitat structure ("vegetation"), in which a *Potamogeton* leaf floating on the water surface was provided as a resting site (Porter [1909] suggested that these are important for the transmission of parasites). We allocated the four treatments haphazardly to one of the compartments in each of three field enclosures, but deliberately varied their relative positions; 20 containers were used in each compartment (giving a total initial sample size of 60 gerrids for all treatments except the triplets, which had 180 bugs). We transferred first instar larvae, within 24 h of hatching, from the laboratory to all four treatments. Within 24 h of the imaginal molt, we

collected the adult gerrids, measured their body length, and dissected them to check for gut symbionts. Due to incomplete temperature records, development times are given in days for this experiment.

Dissections and identification of gut symbionts

To identify symbionts of the digestive tract, we dissected the gerrids under insect Ringer's solution. The alimentary canal was exposed by cutting along the ventral midline from the mesothorax to the seventh abdominal segment (in freshly molted adults, it is easy to tear open the cuticle with fine forceps). The entire gut and the Malpighian tubules could then be removed and placed on a slide in a drop of Ringer's solution. The midgut was teased apart with fine forceps to allow inspection of its contents. We were limited to use of standard light microscopes and thus observed only structural traits; therefore, we were cautious in our identifications, and only indicate genera for the trypanosomatids (Barrington Leigh et al. unpubl.).

Trypanosomatids

In these experiments, we observed trypanosomatid flagellates (Zoomastigina, Trypanosomatidae) of the genera *Blastocrithidia* and *Leptomonas*. These genera are distinguishable morphologically (Wallace et al. 1960, 1965, Wallace 1966). Usually, *Blastocrithidia* occurred in the midgut, and *Leptomonas* in the hindgut. In many of the infected gerrids, trypanosomatids of either genus were concentrated in only a few very dense patches attached to the gut wall, making it impossible to quantify parasite load, or even to achieve reproducible scores on the semi-quantitative scale of Arnqvist and Mäki (1990). Therefore, we only recorded presence or absence of trypanosomatids.

Gregarines

The most conspicuous symbionts were gregarines (Apicomplexa, Gregarinia), possibly of the families Actinocephalidae or Stylocephalidae (Levine 1985). Because of their size, we were able to count them individually. We observed developing gamonts attached to the midgut wall (trophozoites), detached gamonts, and gametocysts; gamonts in syzygy were rare, suggesting that syzygy and the early stages of gametocyst formation take place in a fairly short time. To calculate total gregarine loads, we multiplied the gametocyst counts by two before adding them to the number of trophozoites and gamonts, because pairs of gamonts join to form gametocysts. Because gametocysts, which are shed with the faeces, occur already in fourth-instar larvae, the gregarine loads calculated in this way for teneral adults are conservative estimates of the total load experienced over the entire larval period.

Ciliates

We found ciliates in the hemocoel of many gerrids, but mostly in close association with the digestive tract, i.e., moving on the external surface of the gut and Malphighian tubules. We recorded presence and absence of these ciliates.

Unidentified cysts

In many gerrids, the cells of the Malpighian tubules, hindgut, and to a lesser degree those of the midgut contained cysts, which could not be identified. As these cysts were quite variable, they were often difficult to detect and recognize with certainty, and our estimates of prevalence therefore should be interpreted cautiously.

The gerrids whose offspring were used for the experiments with both the spring and summer generations were kept individually in the laboratory until they died and then dissected (within 24 h of death). As the prevalences and loads of most symbionts were much lower than in other gerrids collected from the wild at the same time but dissected immediately, we suspect that most of them lost symbionts while they were kept in the laboratory. Moreover, many had large amounts of bacteria in their guts or fungal infections of the hemocoel, neither of which we ever observed in gerrids dissected immediately after being collected in the wild. We conclude that these were related to senescence or acquired after death. For these reasons, it was impossible to determine presence or absence or to estimate loads of symbionts from postmortem dissections of these females, and we were unable to examine symbiont effects on reproductive traits.

Statistical analyses

We used a permutation test (Good 1994) for comparisons of development time and adult size in infected and uninfected gerrids. In these tests, the observed differences in averages were compared to a distribution generated under the null hypothesis that the distributions of these life history traits are the same for infected and uninfected bugs.

All correlations reported in this paper are Pearson correlations. Confidence intervals were established by bootstrapping (BC_a method; Efron and Tibshirani 1993).

To assess if individual variation in gregarine load or resistance has a genetic component, we performed an analysis of variance of gregarine load in the first rearing experiment, transformed as $\log(x + 1)$ to reduce skewness. The enclosure in which each bug was reared was included in the model as a fixed effect and sire and dam (nested within sire) as random effects.

Table 1. Prevalences of gut symbionts and their impact on life history parameters in gerrids. The data presented are for the rearing experiment in the spring generation 1994, with a total sample size of 506 individually reared bugs. Mean differences in body length and development time are the signed differences between average values (adjusted for sex and wing morph) for infected and uninfected individuals; e.g., a positive difference indicates that the infected bugs have a higher average value than uninfected ones (overall averages are 273 degree-days for development time and 8.1 mm for length). Statistical significance of the differences between infected and uninfected bugs in mean adult size and development time was established with a permutation test (Good 1994).

Symbiont	Prevalence	Adult body length (mm)		Development time (degree-days)	
		Mean difference	p	Mean difference	p
Blastocrithidia sp.	0.02	−0.16	0.04	13.8	0.02
Leptomonas sp.	0.002	−0.27	0.26	12.6	0.41
Gregarines	0.36	0.01	0.62	−2.2	0.22
Ciliates	0.16	0.01	0.79	−5.8	0.01
Unidentified cysts	0.46	0.00	0.85	−2.6	0.12

Results

The first rearing experiment, in the spring generation, revealed moderate to high prevalences of gregarines and unidentified cysts, and intermediate prevalences of ciliate infections, whereas the trypanosomatids were very rare (Table 1). The trypanosomatids, despite their rarity, had the largest effects on life history traits, reducing body length by 2–3% and increasing development time by ca 5% of the overall average (Table 1). The effects of *Blastocrithidia* sp. on average size and development time were statistically significant in the permutation test. These differences would be nonsignificant after a Bonferroni adjustment to control table-wide significance levels; note, however, that the test has low power because the rarity of the parasite resulted in a highly imbalanced design (i.e., even if not statistically significant at the 5% level, the test still provides fairly strong evidence against the null hypothesis). The effects of *Leptomonas* sp. (Table 1) were similar to those of *Blastocrithidia* sp., but this result must be interpreted with caution because it is based on only a single gerrid that harbored concurrent infections by both these trypanosomatids. The ciliate infections were associated with a small decrease in development time (nominally significant, but nonsignificant after Bonferroni correction), but with no change in adult size. The other symbionts did not affect growth performance.

Gregarine loads in the first rearing experiment ranged from 0 to 429 per gerrid. Even the extremely high loads were not associated with a reduced final size or prolonged development time (Fig. 1). Within each sex and wing morph, the correlations between gregarine loads and adult size or development times were of small magnitude, and all but one of the 95% confidence intervals included zero (Table 2; after Bonferroni correction, none of the correlations is significant). This suggests that gregarine loads did not affect growth performance in the first rearing experiment.

To examine a possible genetic basis of host resistance against gregarines, we performed an analysis of variance with sire, dam, and the enclosure as factors. None of these effects was statistically significant; both the sire and dam(sire) mean squares were smaller than the error mean square. Hence, there is no evidence for genetic variation in host resistance against gregarines. Instead, the variability of gregarine loads reflects random "noise" from environmental variation at a spatial scale smaller than the enclosures.

The second rearing experiment examined whether different environmental factors mediate stronger effects of high gregarine loads on gerrid growth. Adult body size and development time varied significantly among treatments (Table 3), but enclosure effects and the enclosure × treatment interaction were also statistically significant for development time, indicating some heterogeneity in the experimental conditions. Linear contrasts between the control and food limitation treatments were statistically significant for both adult body length ($F = 71.99$; $DF = 1$; $p = 0.0001$; least squares means 7.9 mm and 7.4 mm in the control and food limitation treatments, respectively) and development time ($F = 11.24$; $DF = 1$; $p = 0.0009$; least squares means 28 and 31 d). Therefore, the reduced feeding regime was severe enough to reduce growth performance. Nevertheless, gregarine loads did not differ significantly among treatments, and the estimates of the correlation between gregarine loads and both growth variables were close to zero (Table 4; 14 of the 16 confidence 95% intervals include zero, and for the other two, zero is within the 99% confidence interval). This indicates that the findings of our first experiments also apply under different environmental conditions, including food stress.

Discussion

The results of our experiments suggest major differences between symbionts in their effects on gerrid hosts. The trypanosomatid *Blastocrithidia* sp. had the strongest effects, both prolonging development time and reducing adult size noticeably (the other trypanoso-

matid present in this experiment, *Leptomonas* sp., only occurred in a single gerrid together with *Blastocrithidia* sp., and its effect therefore cannot be assessed). This result complements the findings of Arnqvist and Mäki (1990), who reported negative correlations of trypanosomatid load scores with survival time under starvation and with a measure of locomotor endurance in adult water striders. Unlike most of the gerrid populations studied previously (Laird 1959, Wallace et al. 1960, Tieszen and Molyneux 1989, Arnqvist and Mäki 1990; but see Becker 1923), prevalences of trypanosomatids observed in our study were so low throughout the season that these gut parasites at most can have a marginal influence on the dynamics of the host population.

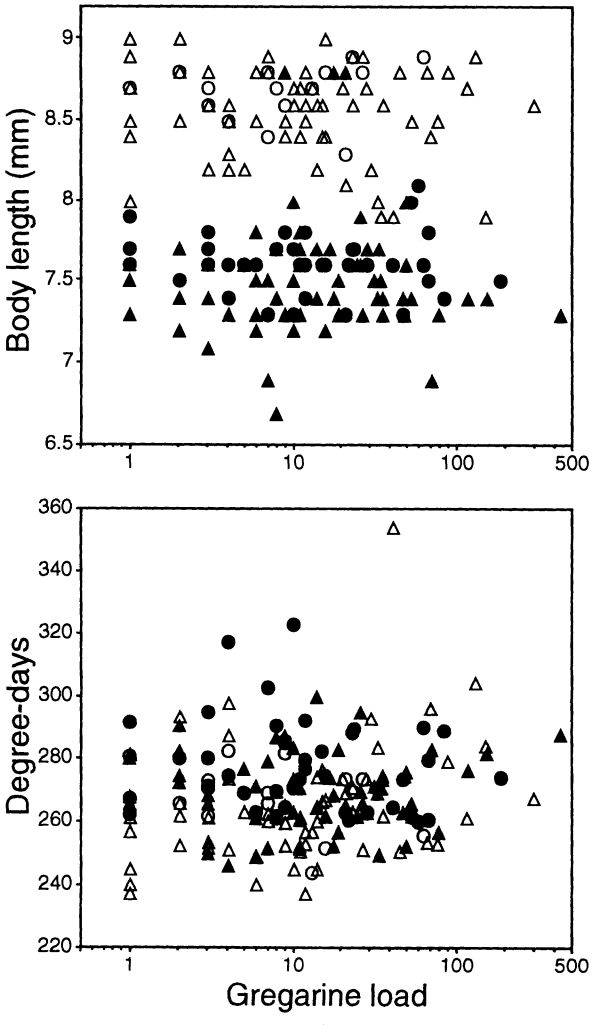

Fig. 1. The relation of gregarine load of water striders to adult body size and development time (in degree-days; spring generation). Gregarine loads are calculated from the combined counts of trophozoites and cysts; they are a conservative estimate of the load during the larval period. Gerrids without gregarines are omitted from the graphs. Symbols: solid symbols, males; open symbols, females; circles, long-winged bugs; triangles, apterous bugs.

Table 2. Correlations between gregarine loads and growth performance in the spring generation. The two growth variables are total body length of teneral adults and development time (in degree-days). The 95% confidence intervals were estimated with the bootstrap (BC_a method; Efron and Tibshirani 1993). Abbreviations for wing morphs are lw for long-winged and ap for wingless (apterous).

Sex	Wing morph	Correlation coefficient	Confidence interval	Sample size
Total body length				
f	lw	0.15	[−0.23, 0.36]	44
f	ap	−0.06	[−0.26, 0.07]	184
m	lw	0.01	[−0.16, 0.25]	92
m	ap	−0.03	[−0.10, 0.11]	179
Development time				
f	lw	−0.28	[−0.43, −0.07]	44
f	ap	0.11	[−0.001, 0.27]	187
m	lw	−0.13	[−0.25, 0.002]	92
m	ap	0.08	[−0.06, 0.19]	180

The ciliates we regularly found in the hemocoel of water striders did not influence adult size, but surprisingly the development time of infected bugs was ca 2% shorter than that of uninfected bugs (Table 1). We doubt the biological relevance of this effect because this difference is less than the intervals between the daily checks for molts; furthermore, it is not statistically significant if a Bonferroni correction is applied to the multiple tests for different symbionts.

Gregarines were present in a large proportion of the gerrids, and often in high numbers. It is surprising that they have only been reported from water striders once (Poisson 1957: 16). Because of their large size, gregarines could not have been overlooked in the studies documenting trypanosomatids (especially the surveys by Tieszen and Molyneux 1989, Arnqvist and Mäki 1990), but were probably absent.

As a consequence of the high loads of large gregarines, often the whole cross-section of the midgut was occluded by gregarines (Fig. 2), and the entire midgut

Table 3. Analyses of variance for adult body length (in millimeters) and development time (in days) in the rearing experiment in the summer generation.

Source	DF	Mean square	F	p
Body length				
Sex	1	55.66	753.32	0.0001
Treatment	3	2.88	38.91	0.0001
Enclosure	2	0.04	0.51	0.60
Treatment × enclosure	6	0.13	1.80	0.10
Error	302	0.07		
Development time				
Sex	1	134.53	7.26	0.007
Treatment	3	165.81	8.94	0.0001
Enclosure	2	102.30	5.52	0.004
Treatment	6	82.55	4.45	0.0002
Error	313	18.54		

Table 4. Correlations between gregarine loads and growth performance the various rearing conditions in the summer generation (all bugs long-winged). The two growth variables are total body length of teneral adults (BL) and development time (DT; in days). The 95% confidence intervals were estimated with the bootstrap (BC_a method; Efron and Tibshirani 1993).

Variable	Sex	Correlation coefficient	Confidence interval	Sample size
Control (standard rearing protocol)				
BL	f	−0.24	[−0.56, 0.12]	20
BL	m	−0.24	[−0.66, 0.32]	30
DT	f	0.14	[−0.23, 0.49]	21
DT	m	0.11	[−0.36, 0.57]	30
Food limitation				
BL	f	0.07	[−0.41, 0.38]	21
BL	m	−0.11	[−0.39, 0.19]	23
DT	f	0.05	[−0.27, 0.30]	23
DT	m	0.15	[−0.29, 0.40]	23
Triplets				
BL	f	−0.03	[−0.19, 0.13]	88
BL	m	−0.31	[−0.57, −0.05]	80
DT	f	−0.11	[−0.24, 0.05]	89
DT	m	0.12	[−0.12, 0.36]	84
Vegetation				
BL	f	−0.06	[−0.45, 0.26]	32
BL	m	0.25	[−0.22, 0.44]	21
DT	f	−0.10	[−0.34, 0.16]	33
DT	m	−0.43	[−0.56, −0.05]	22

of some gerrids was distended by tightly packed gregarines. At least by blocking the gut passage, gregarines must impede food flow and most likely also nutrient absorption in the midgut. Nevertheless, gerrids with gregarine infections scarcely differed from uninfected ones in their adult size or development time in the first experiment (Table 1), and gregarine loads were uncorrelated with both these life history traits (Fig. 1, Table 2).

A similar lack of effects on insect hosts infected by gregarines has also been observed in earlier studies and has led to extensive discussion on the nature of gregarine-host association. Several reports documented damage by gregarines to the gut cells to which they attach, leading to tissue damage (Lipa 1967, Harry 1970, Åbro 1971). As a consequence, there may be reductions in growth (Harry 1970, Zuk 1987, Siegel et al. 1992) or in condition of adults (Zuk 1987, 1988, Simmons 1990). In contrast, Sumner (1936) proposed that gregarine infections have beneficial effects on their hosts (but see the critique by Harry 1967). Still other studies found that the adverse effects of gregarines were increased by food stress and may be overlooked in insects reared under favorable conditions (Harry 1967, Dunkel and Boush 1969).

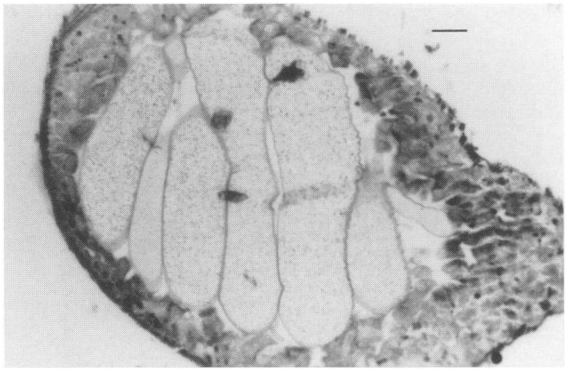

Fig. 2. Cross-section of the midgut of a fifth-instar gerrid with gregarine gamonts filling the entire gut lumen. This section is of a whole midgut fixed in Bouin's fluid, embedded in Paraplast Plus, sectioned at 5 μm, and stained with hematoxylin. The scale bar corresponds to 30 μm.

To examine whether rearing conditions alter gregarine effects on larval life history traits, we conducted experiments with reduced feeding levels, competition, and microhabitat structure. The treatments produced significant differences in development time and adult size; the food limitation treatment in particular reduced growth performance substantially. Still, in none of the treatments was there a clear negative correlation between gregarine load and development time or adult size, and all correlations in the food limitation treatment were close to zero (Table 4). Therefore, our data do not indicate any effects of gregarine loads on larval growth even under adverse conditions. These gregarines appear to be harmless commensals of water striders.

Much of the recent interest in parasites and other symbionts, including those of insects, has been stimulated by theories of host-parasite coevolution, which assume that there is genetic variability in host resistance to pathogens and parasites. Yet relatively few empirical studies have addressed this problem; among invertebrates there is only a handful of studies, mainly from molluscs (Grosholz 1994, and references therein), whereas most studies of insects have focused on resistance against bacterial or viral pathogens (reviewed by Tanada and Kaya 1993: 493–495) or against parasitism from other insects (e.g., Henter and Via 1995). For the protozoan symbionts of interest here, laboratory experiments with bumble bees and a trypanosomatid parasite demonstrated a genetic component of resistance (Shykoff and Schmid-Hempel 1991), but no field studies have been done to assess the variability of natural infections. In our study of water striders, there was no detectable effect of either dam or sire on gregarine loads, despite the fairly large size of the quantitative genetic experiment. The genetic variance, if any, is too small to be detected, given the substantial environmental variability of gregarine transmission; this may stem from spatial variation in the pond microhabitats or from simple stochastic "noise" in the uptake of infective stages (oocysts) by water striders. Therefore, the lack of heritability of resistance limits the potential for coevolution between gerrids and their gregarine symbionts, as does the weak selection indicated by the absence of gregarine effects on growth.

From the results of our experiments, we can rule out protozoan gut symbionts of water striders as an important factor in the dynamics of this study population. Survivorship of larvae reared in enclosures protecting them from predators is high, although infestation by symbionts is comparable to wild bugs in the study population (Barrington Leigh et al. unpubl.). In contrast, predation causes massive mortality, especially in the youngest instars (Spence 1986, Klingenberg and Spence 1996). Moreover, the influence of symbionts on the life histories of surviving bugs is minimal. Trypanosomatids, which are the only symbionts with a substantial effect on gerrid growth (Table 1) and physiological condition (Arnqvist and Mäki 1990), are too rare in this population to account for any more than a trivial fraction of the substantial variation in growth performance (Klingenberg and Spence in press). There are large differences in prevalences of gut symbionts (namely trypanosomatids and gregarines) between our study population and most of those described in the literature. In addition, parasitic mites, which are abundant in other regions and can have a severe impact on infested gerrids (Smith 1989), are absent from our study site. This variation in symbiont communities and in their effects on water strider populations deserves systematic study. Until these factors are better understood, extrapolations from one gerrid population to another should be made cautiously.

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