Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*

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A trade-off between sex and somatic maintenance is fundamental to life-history theory. Tests of this trade-off usually emphasize deleterious consequences of increased reproduction on life span. Here we show the reverse effect, that reductions in the expected life span elevate sexual activity. Experimentally parasitized male *Drosophila nigrospiracula* lived shorter lives, but before dying, they courted females significantly more than unparasitized controls. This greater courtship resulted in increased mating speed, and potentially greater reproductive success than parasitized males would have achieved otherwise. The results show that an environmental reduction in life span increases reproductive effort, and support the hypothesis of a trade-off between these key life-history traits.

Keywords: parasitism; longevity; reproductive effort; *Drosophila*; *Macrocheles*

1. INTRODUCTION

Reproduction, life span, and their interaction are major determinants of fitness in most organisms (Clutton-Brock 1988). To maximize fitness, an individual may decrease current reproductive effort, thereby leading to a longer life and ultimately more progeny (Williams 1966; Gadgil & Bossert 1970; Partridge & Harvey 1988; Lessels 1991). Such a strategy is expected because sexual activity and somatic maintenance may draw from the same limited supply of resources, so that an increase in one should lead to a decrease in the other (Reznick 1985; Bell & Koufopanou 1986; Partridge & Harvey 1988; Lessels 1991). Most of the evidence for this trade-off comes from experiments showing that experimental increases in reproductive effort result in reductions in life span (Partridge & Farquhar 1981; Rose & Charlesworth 1981; Fowler & Partridge 1989; Van Voorhies 1992; Chapman *et al.* 1995; Gems & Riddle 1996). Here we test for linkage between these fundamental life-history traits, wherein expected reductions in longevity cause increased reproductive effort: a causal direction not often explored empirically.

Models on the evolution of life-history strategies for iteroparous organisms, such as *Drosophila*, make the explicit prediction that if adult mortality is high owing to extrinsic factors, the optimum level of reproductive effort at any age will be higher than if mortality were low (Gadgil & Bossert 1970; Charlesworth 1980). This theory may be extended to predict how an individual might alter its tempo of reproduction when the risk of mortality rises. If reproductive effort is normally maintained at submaximum levels resulting from life-history trade-offs, and if resource allocation to reproduction is flexible, then hosts that become infected with deadly parasites should increase expenditure of current reproductive effort (Minchella & Loverde 1981; Forbes 1993).

We experimentally tested this prediction using male *Drosophila nigrospiracula* Patterson & Wheeler and an ecto-parasitic mite *Macrocheles subbadius* Berlese, which co-occur naturally in the North American Sonoran Desert. *M. subbadius* is a cosmopolitan mesostigmatid mite that reproduces on a wide range of ephemeral substrates, including rotting plant tissue, moist soil and animal dung (Krantz 1983). In the Sonoran Desert, *M. subbadius* occupies necroses of saguaro (*Carnegia gigantea*) and cardon (*Pachycereus pringlei*) cacti, which *D. nigrospiracula* uses as oviposition and larval substrates. These mites disperse between substrates by attaching to adult flies; they then use their mouthparts to penetrate the fly integument and feed on haemolymph extracted from the fly's body cavity. Parasitism in this system may reduce host body condition, progeny developmental stability and female fertility (Polak 1996, 1997, 1998). Prevalence of parasitism in natural populations, although highly variable (ranging from 0 to 100%), covaries predictably with phenology of these necrotic cacti (Polak & Markow 1995). In the present study, we first test whether natural levels of parasitism decrease male life span, and hence the opportunity for future reproduction. We then investigate the effect of similar levels of parasitism and male age, and their interaction, on male courtship and mating activity.

2. MATERIALS AND METHODS

(a) *Parasitism and survivorship in males*

Males used in all experiments were from a large, outbred laboratory population derived from nature and maintained for six generations before commencing the work. Flies were cultured in 200-ml culture bottles (containing dry potato flakes, instant *Drosophila* medium, and autoclaved cactus (Polak 1998)) in an incubator with a 12 h light (26 °C): 12 h dark (22 °C) photoperiod and temperature regimen. Males were collected from bottles within 12 h of emergence, and held without females for 48 h in groups of ten individuals in eight fluidram vials containing standard banana–agar medium (Polak 1996). Flies were exposed to parasites for 3 h in chambers (Polak 1996) and sorted: infested males

carried from 1–19 mites (mean±s.e.: 4.4±0.52, $n=76$), and uninfested males were termed exposed controls ($n=39$). Unexposed controls ($n=31$) were kept for 3 h in similar chambers but without mites. Males from each group were placed individually into vials, which were checked daily for dead flies, and live individuals were transferred to a fresh vial every 2–3 d.

Longevity of each male was the number of days from infestation to the last day on which it was seen alive. Mean longevity did not differ between exposed and unexposed controls ($t=1.36$, d.f.$=68$, $p=0.2$), which were therefore pooled. The relationship between thorax length and longevity of unparasitized males was not significant ($F_{1,68}=0.007$, $p>0.9$). In summary, the three experimental populations of males were: (i) unparasitized, control males; (ii) males burdened with one mite; and (iii) males burdened with two or more mites. Survivorship of flies in these populations was fit to the Weibull and exponential functions (Lee 1992), and a one-way analysis of variance was performed on longevity, in which experimental treatment (i.e. mite load) was the independent variable (Zar 1984).

(b) *Parasitism and reproductive effort*

Newly emerged males were held without females in groups of ten individuals at incubator conditions from the time of emergence until they were 4, 14, 34 or 44 days old. Unparasitized male *D. nigrospiracula* reach sexual maturity at 4–5 d after eclosion in the laboratory at 25 °C. During ageing, flies were changed to fresh vials every 2–3 d. Males at each age were experimentally infested (Polak 1996), and same-aged control males were placed in chambers without mites. Thirty-six hours after parasitization, courtship activity was assayed at 25±1 °C. Each male was placed in a separate vial with two females (mated to other males 30 min before the experiment), and the male was allowed to acclimatize to vial conditions for 5 min. These previously mated females were unreceptive, and hence did not permit the experimental male to mate. Each observer simultaneously timed courtship in one infested and one control male from the same age category for 30 min, and the proportion of time spent courting during this interval was calculated. After the courtship assay, mites were counted on males held under a stereomicroscope.

Sample sizes for parasitized and control males were each 50, 45, 25 and 28 in the four age categories (6, 16, 36 and 46 days old), respectively. We pooled results among the two younger and among the two older groups because the results within these categories were qualitatively similar: interaction terms between mite burden and age were not significant in either category ($F_{4,282}=0.18$, $p=0.95$; $F_{5,282}=1.71$, $p=0.14$, respectively). Thus, we present results for two age categories: young (6 and 16 days old) and old (36 and 46 days old). Courtship data were analysed using analysis of covariance with age (i.e. young and old) and degree of parasitism as factors; thorax length (estimate of body size) was entered as a covariate because courtship and thorax length were correlated (slope±s.e: 1.69±0.53, $F_{1,294}=10.26$, $p=0.001$).

(c) *Parasitism and mating latency*

Mating latency was determined in six- and seven-day-old parasitized and control males. Because the presence of four or more attached mites to the male can physically obstruct genital coupling (Polak 1999), latency was measured in males possessing three or fewer mites only. Males were held in vials and infested as described above, and individually aspirated into vials containing two virgin, receptive females; copulation latency was the time
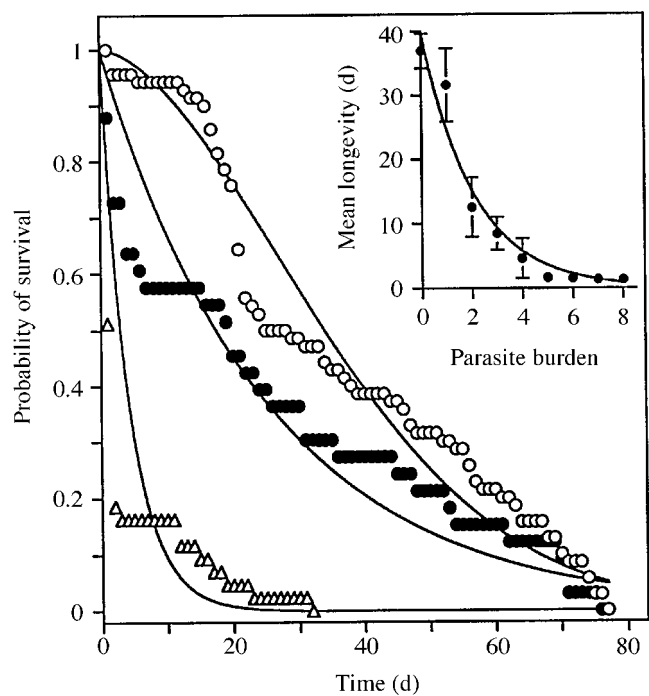

Figure 1. Survival curves for control (open circles), lightly parasitized (filled circles) and heavily parasitized (open triangles) males. The Weibull model, $S(t)=e^{-(\lambda t)^{\gamma}}$, was fitted to the data for control males, whereas the exponential function, $S(t)=e^{-\lambda t}$, was fitted for each of the parasitized groups. Inset shows mean longevity (± 1 s.e.) of males across all treatment categories (one-way analysis of variance, $F_{8,137}=10.25$, $p<0.001$). This relationship is best described by an exponential function (longevity $=34.97 \times 10^{-0.2(\text{mite burden})}$), and emphasizes the dose-dependent response in the dependent variable.

elapsed between the introduction of the experimental male to the onset of copulation. All matings resulted in ejaculate transfer, as determined by dissection of the female in a drop of saline solution under a stereomicroscope. Latency data were $\log_{10}$-transformed to meet assumptions of parametric analysis of variance.

3. RESULTS

The Weibull model, $S(t)=e^{-(\lambda t)^{\gamma}}$, was fitted to survivorship data for control males, whereas data for lightly and heavily infested males were adequately described by the exponential function $S(t)=e^{-\lambda t}$ (figure 1). Estimates of λ for each population indicate a sharply accelerated mortality rate in heavily parasitized flies compared with controls ($\lambda=0.24$ and 0.024, respectively); lightly parasitized males exhibited an intermediate mortality rate ($\lambda=0.040$). Lagrange multiplier χ^2 values for shape (γ of Weibull) indicate a qualitative difference in survivorship of flies with and without mites; this parameter is significant for control flies (H_o, $\gamma=1$, $\chi^2=181.52$, d.f.$=1$, $p<0.0001$), but not for lightly and heavily infested males ($\chi^2=1.45$, d.f.$=1$, $p=0.23$; and $\chi^2=3.51$, d.f.$=1$, $p=0.06$). The inset of figure 1 shows mean longevity of males across all mite treatment categories (one-way analysis of variance, $F_{8,137}=10.25$, $p<0.001$), and emphasizes the dose-dependent reduction in host longevity with mite number. Thus, natural levels of parasitism decrease the male life span, and hence the opportunity for future
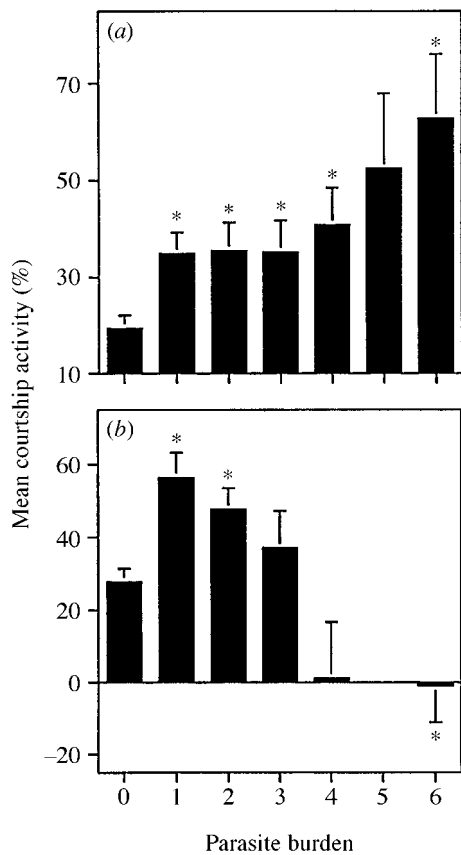
Figure 2. Least-square means in proportion of time spent courting across parasite burdens in young (*a*) and old (*b*) males. Means are from analysis of covariance in which thorax length was entered as the covariate. Error bars represent +1 s.e. and means with an asterisk are significantly different (*p* < 0.05) from the control (zero mites) mean, as determined using the Dunnett testing procedure (Zar 1984). Samples sizes are 95, 38, 21, 17, 12, 3 and 4 for young males carrying 0–6 mites, and 53, 15, 20, 8, 3 and 7 for old males carrying 0–4 and 6 mites, respectively.

reproduction. We tested next the effect of similar levels of parasitism on male courtship and mating activity.

For experiments in which males courted unreceptive females, analysis of covariance revealed a significant effect of parasitism on courtship activity ($F_{6,282} = 5.43$, $p = 0.0001$). Young males in each parasite category augmented expenditure of current reproductive effort, reflected in a 79–223% increase in courtship activity over unparasitized controls; courtship activity increased with parasite burden, demonstrating a dose-dependent response (figure 2). Likewise, older males significantly increased courtship activity under light parasite burdens, but under high burdens courtship fell sharply to values close to zero and significantly below those of controls (figure 2); the interaction between level of parasitism and male age was statistically significant ($F_{5,268} = 5.60$, $p = 0.0001$). We tested whether the observed greater investment in courtship activity resulted in greater mating speed. In nature, male–male competition for receptive females may be intense, whereby males 'jostle' for the opportunity to court and mate with females and where mating speed is probably an important component of male reproductive success (Polak & Markow 1995; Markow 1988). Parasitized males exhibited significantly
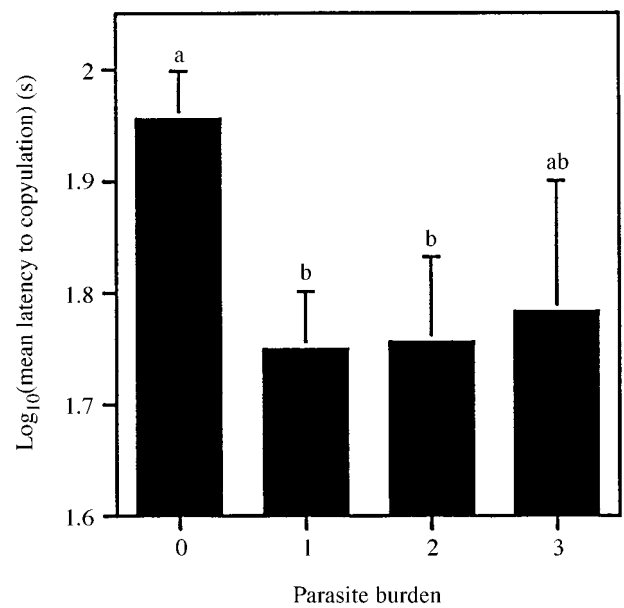

Figure 3. Mean (+1 s.e.) latency to copulation (mating speed) with virgin females across mite treatment categories. Means sharing a letter are not significantly different at the $\alpha = 0.05$ level. Sample sizes are 58, 39, 23 and 12 for males carrying 0–3 mites, respectively.

shorter latency to copulation with virgin females than controls (one-way analysis of variance, $F_{3,128} = 3.75$, $p = 0.013$) (figure 3), indicating that the increased allocation to courtship may lead to reproductive gains.

4. DISCUSSION

The results show that parasitism *per se* causes a dose-dependent drop in life span, probably resulting from mechanical injury and nutrient extraction by the parasite (Polak 1996, 1998). Despite these ill effects, and the fact that courtship and mating are probably energetically costly (Partridge & Farquhar 1981; Partridge 1988), flies responded to parasitism by increasing reproductive performance, and in younger males courtship increased with parasite burden. Older males significantly increased courtship duration at light burdens, but courted markedly less than controls when heavily parasitized, an effect probably resulting from the joint influence of parasitism and loss of physiological vigour due to old age. The demonstrated association between elevated courtship activity and mating speed ascribes adaptive value to this behavioural plasticity.

It is possible, however, that the increased expenditure of reproductive effort results from parasite manipulation of host behaviour to increase parasite fitness (Ewald 1994). For example, sexually transmitted parasites may enhance their rate of transmission by increasing the mating propensity of their host. However, the available evidence indicates that *M. subbadius* is not sexually transmissible. Of the numerous copulations witnessed in the laboratory in this and previous studies (e.g. Polak & Markow 1995), no female (or male) acquired a mite from a parasitized partner during any phase of mounting or copulation. Although a mite may parasitize multiple hosts during its lifetime, mites must return to the substrate from the present host before parasitizing a

subsequent fly. Thus, we favour the interpretation that this behavioural switch is an adaptive strategy of the host organism, and not an outcome of the parasite manipulation of host sexual behaviour to promote the parasite's transmission between hosts.

To our knowledge the only other example of reproductive compensation in the face of parasitism involves laboratory genetic strains of the snail *Biomphalaria glabrata*. Snails increased egg laying upon exposure and infection by the castrating trematode *Schistosoma mansoni* (Minchella & Loverde 1981; Thornhill *et al.* 1986), although for unknown reasons this effect was not detected in a study of another snail, *Biomphalaria pfeifferi* (Meuleman 1972).

The physiological mechanism linking variations in parasite burden to reproductive effort in *Drosophila* is yet unknown, but may include the induction of haemolymph effectors in the fly, which either directly or indirectly modulate nervous system activity (Beckage 1997). In our experiments we used an extrinsic source of mortality to manipulate the life span in males randomly selected from a large, outbred population. Thus, this behavioural plasticity probably does not reflect a genetic trade-off based on the pleiotropic action of genes (Templeton & Johnston 1982), as revealed, for example, in *Drosophila melanogaster* by genetic manipulation of the life span through artificial selection (Rose & Charlesworth 1981).

The switch towards higher reproductive effort in *D. nigrospiracula* appears adaptive, and mirrors how a population might respond evolutionarily. In environments with high and constant extrinsic mortality, genotypes will be selected to produce more offspring sooner, in part because the corresponding costs of elevated reproduction (in terms of lost future mating opportunities) are low anyway as a result of environmental effects (Gadgil & Bossert 1970; Michod 1979; Reznick 1985; Partridge & Harvey 1988). Parasitism in *D. nigrospiracula* appears to offset these costs similarly, thereby facilitating this behavioural switch. However, because rates of parasitism are highly variable in nature (Polak & Markow 1995), an evolutionary strategy of invariable high effort, although advantageous in times of high parasite pressure, would probably be negatively selected overall. Our results suggest that the optimal strategy in the face of such potent but variable selection is a conditional one, resulting in the upregulation of reproductive effort in relation to the probability of death.

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