Flight behaviour attenuates the trade-off between flight capability and reproduction in a wing polymorphic cricket

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Flight-dimorphic insects have been used extensively to study trade-offs between energetically costly traits. Individuals may develop and maintain structures required for flight, or alternatively they may invest in reproduction. Previous experiments have not examined whether flight itself might affect investment into reproduction. As in other *Gryllus* species, flight-capable individuals of the wing polymorphic cricket, *Gryllus texensis*, incur an apparent reproductive penalty for being able to fly, expressed as smaller ovaries in females and lower courtship propensity in males, than their flight-incapable counterparts. We find that a short bout of flight eliminates the trade-off. Two days after the flight, the ovaries of flight-capable females were comparable with those of short-winged females. Similarly, flight markedly increased the probability of courtship behaviour. Our results suggest that the impact of the flight-reproduction trade-off described in earlier studies may have been overestimated.

Keywords: life-history trade-off; flight behaviour; flight dimorphism; wing polymorphism; ovarian development; courtship behaviour

1. INTRODUCTION

Life-history theory predicts that when different traits are energetically costly, some traits may be emphasized at the expense of others (Roff 1986; Zera & Denno 1997; Zera & Harshman 2001). A well-established trade-off exists between flight ability and reproduction. This occurs in many insect taxa, and has been studied particularly intensively in wing-dimorphic field crickets (Harrison 1980; Roff 1986; Zera & Denno 1997). One morph is flight capable, possessing long hind wings, well-developed flight musculature, and metabolic pathways to produce flight fuel. In females of this morph, ovarian growth is delayed, resulting in longer reproductive latency and lower early adulthood fecundity relative to an alternative, flight-incapable morph that has short hind wings, poorly developed flight muscles and metabolic pathways that favour yolk production. Similarly, flight-capable males invest less than flight-incapable males into production of a mate attraction song (Crnokrak & Roff 1995; but cf. Bertram 2007) and courtship behaviour (Guerra & Pollack 2007). Flight-capable individuals may, later in

life, lose this ability and divert resources to reproduction (Roff 1986; Zera & Denno 1997; Guerra & Pollack 2007). Although a large body of work has investigated the differences in reproductive investment between flight morphs, in these experiments individuals were not flown; thus the effect of flight on this life-history trade-off was not examined (cf. Zera 2005).

Flight has been shown to have both positive and negative effects on the reproductive output of insects. Flight enhances oviposition in the wing monomorphic migratory grasshopper, *Melanoplus sanguinipes* (McAnelly & Rankin 1986). By contrast, flight reduces egg production in the wing monomorphic fruitfly, *Drosophila melanogaster* (Roff 1977). In males, flight enhances mating behaviour in the wing-monomorphic cricket, *Gryllus bimaculatus* (Dyakonova & Krushinsky 2008). In a wing-polymorphic cricket, *Gryllus texensis*, males captured after having been observed flying in the field had enhanced calling behaviour relative to field-captured, short-winged males (Bertram 2007). To test whether flight modulates the trade-off between flight ability and reproduction, we measured the consequences of short bouts of tethered flight for both ovarian development and male courtship behaviour in *G. texensis*.

2. MATERIAL AND METHODS

Virgin *G. texensis* were obtained from an established laboratory colony. Animals were reared with ad libitum access to food and water, as described in Guerra & Pollack (2007). Long-winged crickets, aged 3–5 days after the last moult, were attached to a wooden applicator stick at the pronotum using wax and placed in front of a small fan to promote flight. Flight was terminated after 5 min. As a handling control, crickets were treated as above except that instead of flying they were placed on an air-supported styrofoam ball (which permitted tethered walking) for 5 min. Treatments were performed under dim red light, within the first 4 hours of scotophase, as this is when flight is most common in the field (Cade 1979b).

(a) Effect of flight on ovarian development

Two days after treatment, individuals were dissected to inspect their flight muscles and weigh their ovaries. Unflown animals were examined at equivalent ages, i.e. 5–7 days after the adult moult. As an allometric control, we divided the combined fresh weight of both ovaries by the pre-dissection weight of the female. Flight muscles were classified as functional or non-functional according to colour and size (large and pink, or small and white, respectively; Shiga *et al.* 1991; Guerra & Pollack 2007).

We log transformed the measurements of both body weight and ovarian weight before statistical tests, to fulfil assumptions for normality. Normalized ovary weight remained non-normal even after transformation (arcsine), so we analysed these data using non-parametric tests.

(b) Effect of flight on male courtship behaviour

We tested only long-winged males (5–13 days after last moult), as Guerra & Pollack (2007) previously found that flight-capable males had lower courtship propensity than short-winged individuals. The courtship protocol was identical to that described by Guerra & Pollack (2007). Briefly, a single male was placed in an arena with a single long-winged female (aged 7–9 days after the moult to adulthood). If no contact occurred within 6 min, the trial was discontinued. Otherwise, the trial continued either until the male began to court the female, as indicated by the production of courtship song, or until 5 min elapsed with no courtship display, in which case the male was scored as non-courting. Trials were performed under dim red illumination.

To determine the effect of flight on male courtship behaviour, flown males were tested in courtship trials either immediately following flight, i.e. during the first 4 hours of scotophase, or 10 hours later, shortly before subjective dawn. This delay was introduced because field studies show that flight is most common early in the evening, but mating is most common at around dawn (Cade 1979a). Handling controls were treated according to the same time regimes. Flight-muscle condition of the males was

determined following the courtship trials. We used two-tailed Fisher's exact probability tests (Bonferroni-corrected when necessary) to compare frequencies of courtship behaviour.

3. RESULTS

There were no differences among the different types of control, i.e. handled versus unhandled females (raw ovary weight: $t_{21} = -0.093$, $p = 0.9267$; normalized ovary weight: $t_{21} = -0.175$, $p = 0.8627$), and males that were unhandled, handled and then tested immediately, or handled and tested after a delay (Fisher's exact probability test, $p > 0.99$). We therefore combined data from the control groups within each of these experiments.

(a) Effect of flight on ovarian development

Body weight, measured at ages 5–7 days, did not differ between experimental groups ($F_{2,44} = 1.78$, $p = 0.180$; mean $\pm$ 1 s.d.: control long winged = 447 ± 81 mg; flown long winged = 471 ± 92 mg; short winged = 410 ± 91 mg). All long-winged females ($n = 33$), regardless of treatment, had functional, pink flight muscles. All short-winged females ($n = 14$) had non-functional, white flight muscles.

We measured ovarian weight as an indicator of investment into reproduction. Earlier work (*Gryllus firmus*; Zera *et al.* 1997) showed that this is a reliable proxy for the number of post-vitellogenic eggs, and we confirmed this for *G. texensis*: four out of five unflown long-winged females had no mature oocytes (defined as length of 0.5 mm or more) and the fifth had only two, whereas all of five flown females had mature oocytes, with counts ranging between 22 and 140. Ovary weight varied with treatment (figure 1a; $F_{2,44} = 69.78$ mg, $p < 0.0001$). Short-winged females had larger ovaries than long-winged controls, and flight caused an increase in ovarian weight (flown versus control long-winged females, Tukey HSD, $p < 0.05$ for both comparisons), such that by 2 days after the flight, ovary weight was similar between long-winged females that had flown and short-winged females (Tukey HSD, $p > 0.05$).

The same result was found when ovary weight was normalized by body weight (Kruskal–Wallis: $\chi^2 = 35.55$, d.f. = 2, $p < 0.0001$; figure 1b). A *post hoc* Dunn's test ($\alpha = 0.05$) showed that the normalized ovary weight of short-winged and long-winged, flown females were similar, and that both were greater than that of controls.

(b) Effect of flight on male courtship behaviour

All males had functional, pink flight muscles. Flown males courted with higher probability than controls, whether tested immediately after flight or 10 hours later (figure 2; Bonferroni-corrected Fisher's exact probability tests, $\alpha = 0.017$, $p < 0.0001$ in both cases). There was no difference between males tested immediately after flight and those tested 10 hours later ($p > 0.99$).

4. DISCUSSION

We show that in *G. texensis*, a species in which there is an oogenesis–flight trade-off in females (our data) and a lower probability of courtship behaviour in

Figure 1. (a) Raw ovarian weights of the different treatment groups (mean $\pm$ s.d.). (b) Normalized ovarian weights for the different experimental groups. The line across the middle of each box is the median value; ends of the boxes represent quartiles; vertical lines show ranges of values.

Figure 2. Effect of flight on male courtship singing propensity. Sample sizes: control, $n = 24$; experimental groups, $n = 8$ for each.

flight-capable males relative to flight-incapable males (Guerra & Pollack 2007), a single, short flight bout can mitigate the reproductive penalty of being flight capable. In males, this effect can last at least overnight, in accord with the delay in mating relative to flight that has been observed in the field (Cade 1979a,b). The enhancement of reproductive output can occur without any obvious changes in flight muscle condition; both males and females still had functional flight muscles when reproductive enhancements were demonstrated, at least as indicated by muscle colour and size. This suggests that energy resources are sufficient to support both reproduction and flight ability, at least in our well-fed, laboratory reared animals. It remains to be seen whether flight would accelerate reproduction under less favourable dietary conditions. Indeed, earlier work has shown that the trade-off between flight capability and investment in reproduction is amplified under conditions of dietary restriction (Zera *et al.* 1998).

Whether our findings relate to the occurrence of a trade-off between flight and reproduction in the field is unclear. Virtually nothing is known about the age of onset, frequency or duration of flights in the field. However, our results for males are consistent with previous work on *G. texensis*, which showed that calling behaviour is enhanced in males that were observed to have flown, relative to flight-incapable males (Bertram 2007). Similarly, our findings for females are consistent with those for other insect taxa, where reproduction commences after a dispersal flight (e.g. aphids: Kennedy & Booth 1963; ants: Hölldobler & Wilson 1977; migratory grasshoppers: McAnelly & Rankin 1986; water striders: Kaitala & Huldén 1990; see Roff & Fairbairn 2007 for review). In contrast to these examples, Zera *et al.* (2007) found that field-collected, flight-capable females of *G. firmus* had smaller ovaries than short-winged females or long-winged females with histolysed muscles. Although these results are intriguing, flight history (as distinct from flight capability) was not known for most animals, nor was it known whether the captured animals had already oviposited. Additional field data are required to determine the impact of flight on reproductive output in crickets.

Evolutionary biologists have considered flight dimorphism to result from a balance in costs and benefits to each of the alternative flight morphs (Roff 1986). Much of the evidence supporting this view comes from studies on crickets, in which flight ability is assumed to carry the benefits of dispersion (e.g. moving to a more suitable habitat), at the cost of decreased reproduction relative to flight-incapable individuals. Our findings suggest that the reproductive penalty of flight ability may have been overestimated. We suggest that other potential costs associated with flight (as opposed to merely being flight capable) require further investigation, most importantly under field conditions.

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