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Male field crickets infested by parasitoid flies express phenotypes that may benefit the parasitoids

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Parasites can cause changes in the phenotypes of their hosts that may benefit the parasite, the host, or both. To understand the evolutionary dynamics of host–parasite interactions it is necessary to first examine the effect of parasitic infestation on the host phenotype and whether the host or parasite benefits from these changes. The fly *Ormia ochracea* parasitizes the variable field cricket, *Gryllus lineaticeps*, and it uses male song to locate hosts for its lethal larvae. Adult flies preferentially orient to male songs with faster and longer chirps. We tested the effect of larval infestation on two types of host traits. First, we tested whether infestation affects male singing activity and song characters. Infested males were significantly less likely to sing than noninfested males, and when they did sing, they sang less frequently. Infestation thus reduced a male's ability to attract mates, which may benefit the parasitoid if mating activity increases predation, superparasitism and/or energetic costs for their hosts. No song character we measured, however, differed between infested and noninfested males. Second, we tested whether infestation affects host mass. Infested males gained more mass than noninfested males, which was not explained by the reduced singing of infested males. Importantly, parasitoids that developed in males that gained more mass were heavier as pupae, which may increase their viability and reproductive success as adults. These changes in the host may be beneficial side-effects of the pathology of parasitism, the result of a host-compensatory response, or the result of host manipulation by the parasitoid.

Animals that are infected by parasites often differ from uninfected animals in their behaviour, morphology and/or physiology (Thomas et al. 2005). Some of the most spectacular changes in host phenotype include the expression of submissive behaviour (Libersat et al. 2009), host paralysis (Piek et al. 1971), induced suicide (Hohorst & Graefe 1961; Moore 1995; Biron et al. 2005), the building of safe pupation sites for parasitoids (Eberhard 2000, 2001), changes in host coloration to mimic a food item of a subsequent host (Yanoviak et al. 2008) and/or changes in morphology to attract predators (Wesenburg-Lund 1931; Kagan 1951). The phenotypic consequences of parasitism could be host manipulations caused by traits encoded in the parasite's genome (i.e. the 'extended phenotype' hypothesis; Dawkins 1982), or fortuitous by-products of infection that may result in benefits for the parasite (Poulin 2010). Recently, it has been suggested that both the parasite and the host may gain benefits if host changes mitigate the costs of infection for the host and concomitantly increase the parasite's transmission rate (Lefèvre et al. 2009), or if the parasite forces the host to collaborate (i.e. 'mafia-like' manipulation; Zahavi 1979; Thomas et al. 2005; Lefèvre et al. 2009). Changes of the host could also represent host adaptations for resisting or coping with parasites (e.g. Poulin et al. 1994; Wellnitz 2005; Poulin 2010). Finally, changes in the host phenotype may be the product of pathological side-effects of infection that are nonadaptive for either side (Minchella 1985). However, it has been argued that pathological side-effects that increase the reproductive success of the host and/or parasite will not be selected against (Combes 2001; Moore 2002; Klein 2005), and, if they have a genetic basis, may become adaptive (Poulin 2010).

Which side of the parasite–host interaction benefits from the changes in the host phenotype is often not clear and is the subject of an ongoing debate (e.g. Poulin 1995, 2010; Thomas et al. 2005; Lefèvre et al. 2009). The mechanisms mediating changes in the host are often highly complex, making it difficult to identify which side is responsible for the changes and who benefits from them (Lefèvre et al. 2009). Additionally, it is difficult to distinguish between some of the alternative explanations for host changes. For example, some cases of host changes have been interpreted as the result of manipulations sensu stricto (Dawkins 1982) or adaptive host responses, whereas these cases could also be interpreted as parasites exploiting the host compensatory response to parasitism

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(Lefèvre et al. 2009). Nevertheless, the first step to understand the dynamics of the parasite–host relationship is to determine whether the host phenotype changes as a result of parasitism, and whether these changes are beneficial for the parasite and/or the host.

The tachinid fly *Ormia ochracea* is a parasitoid that uses field crickets as hosts. Its larvae live and develop inside the host and kill the host when they emerge and pupate into free-living adults (Adamo et al. 1995b). *Ormia ochracea* ranges in North America from Florida to California and Hawaii and it parasitizes at least six species of field crickets across this range (Cade 1975; Walker 1986; Walker & Wineriter 1991; Zuk et al. 1993; Wagner 1996; Hedrick & Kortet 2006). In different geographical regions, the fly uses a different species as a host for its larvae. It locates its hosts using the mating songs of male crickets, and male parasitism rates can be as high as 80% in some species (Cade 1975). Once the fly lands near a male cricket, it expels two to three planidial larvae on the male and approximately six larvae on the ground around the male (Adamo et al. 1995a). Once the larvae make contact with a cricket, they burrow into the cricket's body and develop for the first 3 days within the thoracic flight muscles (the first phase of infestation) before they move to the abdomen (the second phase of infestation) to continue their development (Adamo et al. 1995b). Tissue damage due to larval feeding takes place only during the second phase of the infestation and primarily targets thoracic and abdominal muscles and fat tissue (Adamo et al. 1995b). The larvae emerge from the cricket approximately 7 days after infestation and kill the host during this process (Adamo et al. 1995b). After emergence, the larvae pupate and then eclose into adult flies.

We examined the effects of larval infestation on the behaviour and morphology of male variable field crickets, *Gryllus lineaticeps*. This cricket species is a major host for California populations of *O. ochracea* (Wagner 1996; Wagner & Basolo 2007a; Martin & Wagner 2010). We specifically examined changes in host traits that should affect the fitness of the parasitoids. First, we tested whether infestation with *O. ochracea* larvae influences male singing activity and song characters. Changes in male song may be beneficial for the larvae in the context of superparasitism (i.e. infestation of a previously infested host; Fiske 1910). Larvae that parasitize a cricket within 24 h of the initial infestation incur 100% mortality (Adamo et al. 1995a), and the initial residents may experience increased competition, which could influence their size and, thus, fitness (see below). There is no evidence that the flies can distinguish between parasitized and nonparasitized crickets using nonacoustic cues (Adamo et al. 1995a). However, the flies usually prefer the same song types that female crickets prefer (e.g. Wagner 1996; Gray & Cade 1999; Wagner & Basolo 2007a, b), and larval infestation may cause changes in host singing activity or song characters that reduce the probability of a subsequent infestation by other flies. In addition, changes in singing activity or song characters may reduce host energy expenditure (Hoback & Wagner 1997) and the risk of attracting predators.

Second, we tested whether the fly larvae cause changes in host mass, and whether pupal mass is affected by changes in host mass. Since the larvae develop inside the host, host size may determine the amount of food available to the larvae and, thus, pupal size (Welch 2006). Pupal size has major effects on a fly's fitness: bigger pupae have greater survival and develop into bigger adults, which may have higher fecundity (e.g. King 1993; Adamo et al. 1995b; Allen & Hunt 2001; Kolluru & Zuk 2001). The parasitoids could affect host size through at least two mechanisms: parasitism could result in reduced energy expenditure (e.g. in a reduction in singing and other costly activities) or in increased foraging activity.

METHODS

Study Animals

We collected adult female *O. ochracea* at Rancho Sierra Vista in the Santa Monica Mountain National Recreation Area (near Newberry Park, CA, U.S.A.) in the summer of 2010, using broadcasts of *G. lineaticeps* song (Wagner & Basolo 2007b). The flies were brought to the University of Nebraska-Lincoln for experiments. The flies were kept in individual containers (13 × 17 × 8 cm) and fed with applesauce (Best Choice™, Ft Worth, TX, U.S.A.) and cotton (Padco™, U.S. Cotton (Canada) Co., Lachine, Québec, Canada) soaked with a saturated sugar solution until the start of experiments. The fly food was replaced every 2 days.

We collected adult female *G. lineaticeps* from the same site as the flies in the summer 2008 to establish laboratory populations. Most of the female crickets mated before capture in the field and laid fertile eggs in the laboratory. Individuals hatching from those eggs constituted the first laboratory generation. We actively managed pairings between males and females for subsequent laboratory generations to reduce inbreeding. We used males of the second and older laboratory generations in our experiments.

Crickets were reared to adulthood using the protocol described in Beckers & Wagner (2011). In brief, last-instar juvenile males were placed into individual containers and checked daily for adult moult. Individual containers had a paper towel substrate and cardboard shelters and the crickets were provided with water and cat chow (Nestlé, Purina PetCare Co., St Louis, MO, U.S.A.) ad libitum. We kept all adult males until their death in environmental chambers set to a 14:10 h light:dark cycle at an ambient temperature of 21.1–27.2 °C and a relative humidity of 33–70%.

Infestations

We artificially infested crickets to examine the effects of the parasitoid larvae on cricket singing behaviour and mass. Crickets were 7–12 days of adult age at the beginning of the experiments. We randomly assigned males to one of two treatment groups: infested ($N = 27$) and noninfested ($N = 26$). The age of the males did not differ significantly between treatment groups (infested: average ± SE: 9.07 ± 0.287 days; noninfested: 8.88 ± 0.325 days; Mann–Whitney U test: $U = 673$, $P = 0.605$). Males tested were drawn from 19 full-sibling families. We used no more than two males from the same family for either treatment (on average, infested: 1.4 males/family; noninfested: 1.3 males/family).

We killed each fly by removing its head and then dissected its abdomen to obtain planidial larvae for the infestation of the male crickets (for a detailed description see Vincent & Bertram 2009). On the day of infestation, we weighed the crickets and used a probe to transfer larvae to the crickets. Larvae were deposited on the dorsal surface of the cricket, along the membranous area between head and thorax (Vincent & Bertram 2009). We transferred three larvae to each cricket, which corresponds to a natural density of larvae found in cricket hosts infected by *O. ochracea* (1–3 larvae; Adamo et al. 1995a; Kolluru & Zuk 2001). Since larvae can move around on the cricket and may not successfully enter the host (Vincent & Bertram 2009), the number of larvae that emerged from some crickets was lower than the number transferred. However, larvae emerged from all infested crickets and all infested crickets died 7–10 days after initial infestation. Between two and three larvae emerged from most of the infested crickets. In two cases, four larvae emerged, which could be explained by errors in the number of larvae transferred. We included these individuals in our analyses, which did not change our results. Crickets from the noninfested treatment were handled in the same way as the infested crickets