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Non-random mating in the beetle *Cryptocephalus hypochaeridis*

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Abstract

Non-random mating has been observed in many species of beetle, where larger or heavier males have a greater mating success. This difference in male mating success could be through direct competition between males, or female choice. We examined non-random mating in the leaf beetle *Cryptocephalus hypochaeridis* (L.) (Coleoptera Chrysomelidae). In the field, we found that successfully mating males were significantly heavier than unsuccessful males. We also found that the flowers in which we found beetles were significantly taller and wider than unoccupied flowers. However, we found no relationship between flower morphology and the mass of male occupants, suggesting that females are actively choosing the larger males. In the laboratory, females were found to show no preference for male size, and mated randomly. This suggests that mate choice in *C. hypochaeridis* is dependent upon cues other than flower size and height or male mass. We discuss what these cues might be, and how our results relate to the mating strategies of chrysomelid beetles. We also describe the mating behaviour of *C. hypochaeridis*.

Key words: sexual selection, *Leontodon hispidus*, *Cryptocephalus hypochaeridis*, leaf beetle, Chrysomelidae, mate choice, courtship.

Introduction

The two most widely studied selective processes in sexual selection are female choice and male-male competition (Andersson, 1994). Both processes can be involved in determining which male a female will eventually mate with. As a result, if a trait differs between individuals and is shown to be correlated with their mating success, it is not necessarily obvious which selective process is operating: for example, males with large weapons may actively compete for access to females, or instead may not fight, and simply be picked out by choosy females on the size of their unused weapon. We therefore require detailed observations to identify how selection is working within a species.

In insects, female choice is typically based upon a brief period of courtship, where individual differences in behavioural and physical cues contribute to non-random matings (Thornhill and Alcock, 1983; Choe and Crespi, 1997). In beetles, non-random mating has been explored in a range of species, and male mating success has been correlated with attributes such as body colour (Majerus *et al.*, 1982; Wang, 2002), body size (Hanks *et al.*, 1996; Wang, 2002), and body mass (Tomlinson *et al.*, 1995). In this study, we investigated mate choice by female *Cryptocephalus hypochaeridis* (L.) (Coleoptera Chrysomelidae), a leaf beetle commonly found in southern England (see Piper and Compton, 2003; Leonardi and Sassi, 2001, note that several morphotypes exist in Europe, but only one form is found in the British Isles). We examined whether *C. hypochaeridis* exhibits non-random mating with respect to male body mass, size, and location in the field, and with respect to male body mass in laboratory experiments.

Materials and methods

Sexual dimorphism and flower preference

Beetles were observed and collected on 25th-30th June 2004 at two locations near Dorking, Surrey, UK: the Gallops (a chalk meadow, Ordnance Survey national grid reference TQ179534) and a woodland clearing in Juniperhill Wood (TQ178531). The beetles were conspicuous on yellow flowers, particularly *Leontodon hispidus* L. and species of *Ranunculus* L.. Collected beetles were housed individually in petri dishes or specimen tubes, each with a similarly-sized *L. hispidus* flower for food.

Preliminary observations established that the beetles could easily be sexed based upon the shape of a bowl-shaped depression on the ventral surface of the penultimate abdominal segment, where the female depression was visibly deeper and narrower than that of the males. Each individual's mass was taken (to 0.1 mg) after individual beetles had been exposed to carbon dioxide for the minimum amount of time necessary to temporarily immobilise them. We also compared male and female mass for dimorphism using a two-sample t-test assuming unequal variances.

We assessed whether the beetles showed a preference for particular types of flowers by sampling all the individuals found within five 30 × 1.5 m transects arrayed in parallel along our sites. We recorded the total number on four genera of locally flowering plants (*L. hispidus*, *Ranunculus* sp., and *Hypericum* sp. – each with yellow flowers – and *Ligustrum vulgare* L., with white flowers). When a beetle was found on a flower, we recorded the flower species, the diameter of the flower, and its height from the soil surface. We also recorded the diameter and height of the nearest unoccupied flower of the same species. We conducted paired t-tests to assess whether the beetles showed a preference for specific flower heights and diameters (where flower heights were log-transformed to satisfy statistical assumptions of normality), and assessed whether there was a relationship between height and diameter of occupied flowers using Pearson's correlation test.

Field mating preferences

Twenty-five randomly sampled mating pairs were collected from *L. hispidus* flowers, together with each mating pair's closest non-mating beetle. We then compared the mass of the mating and non-mating males for 18 of the pairs (the single beetle was female in the other paired samples) – the males and females from these 18 pairs were saved and used in the laboratory experiment described in the next section. For the 12 of these pairs where both mating and non-mating males were found on *L. hispidus* flowers, we also recorded and compared the diameter and height of the flowers each male was found on.

Laboratory mating preferences

We assessed female mate choice on two consecutive days by placing the 18 females collected above individually in 5 cm petri dishes, and presenting each one with two males: one randomly selected from the 18 lightest males captured above, and one randomly selected from the 18 heaviest. Each of the 18 females and 36 males was used in both days of experiments, and the random allocation to pairs was controlled so that males did not face either males or females they had been paired with on the first day. The difference in the masses of the males in each experiment ranged from 1.6 to 5.3 mg (which is within the range of differences between males found in the population – see below). These experiments were then observed for six hours. When a mating occurred that lasted at least one minute, we removed the unmated male from the Petri dish. After the beetles stopped mating, we weighed both males to establish whether the heavier or lighter male had mated. There was almost invariably some mass loss during the experiment (no food was provided), but this loss was small in comparison to the initial mass difference between males, and so unequivocal identification of which of the males had mated was still possible. We repeated observations on the same individuals, and some of these females did not mate on one or both days (13 mated on the first day, and 11 on the second); to avoid pseudoreplication, we therefore tested for a difference in the mass of the mated and unmated males using paired *t*-tests separately for each day.

Results

Mating behaviour

During a typical mating, the male mounted the female from behind, clasping the underside of her terminal abdominal segment with his hind tarsi, leaving his front legs free. He extended his aedeagus and attempted to insert it into the female reproductive tract. The female was then observed shaking her abdomen vigorously from side to side in up to six bursts of five to ten seconds in length (possibly as a mechanism for avoiding copulation, or as a mate selection behaviour). If the male was able to maintain his grip, he coupled his aedeagus with the female, keeping his body vertically perpendicular to her, in a pose similar to that of the coccinellids (Mike Majerus, *pers. comm.*), and thrusting his abdomen two or three times in succession at regular intervals. At irregular intervals throughout copulation the male was observed palpating the female's elytra with his antennae for three to six seconds, accompanied by five to seven vigorous abdominal thrusts. Also at irregular intervals, the female clasped the male's aedeagus with her hind tarsi and ran them along it. Females continued to feed and walk around during mating. After mating, females were observed to lay their eggs in the base of the host flower.

Copulation ended when either the female dislodged the male by abdominal shaking, or when he dismounted. Matings where the male dismounted of his own accord lasted from six to 449 minutes (mean time ± S.E. = 143.3 ± 27.2 minutes, $N = 22$).

Sexual dimorphism and flower preference

Females were significantly heavier than males (mean female mass ± S.E. = 22.89 ± 0.56 mg, $N = 22$; male mass = 16.68 ± 0.16 mg, $N = 95$; $t_{24.46} = 10.69, P < 0.001$).

Compared with unoccupied flowers, beetles were found in flowers with a wider diameter (figure 1a; $t_{60} = 7.10$, $P < 0.001$) and that were taller (figure 1b; $t_{60} = 5.76$, $P < 0.001$). This was also true if we considered *L. hispidus* alone (height: $t_{47} = 5.26$, $P < 0.001$; width: $t_{47} = 6.32$, $P < 0.001$). Considering the pooled data, there was no correlation between flower height and diameter ($r = -0.085$, $P = 0.513$).

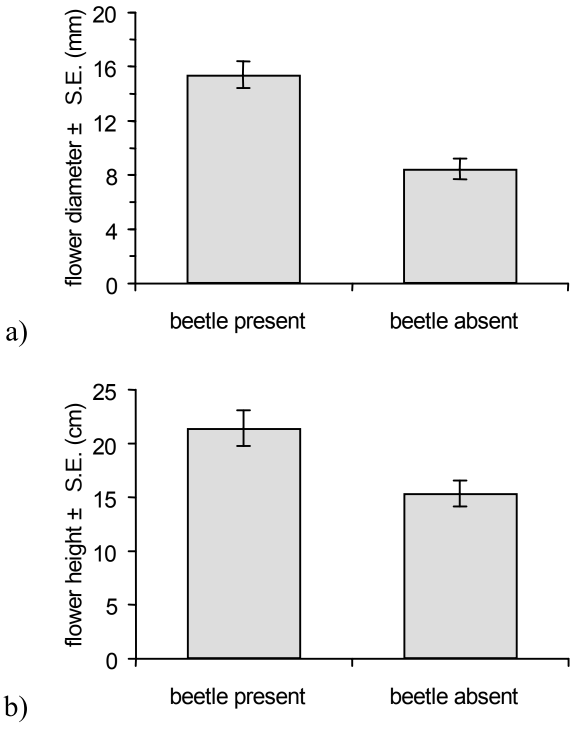

Figure 1. Figure showing that (a) the mean diameter of all flowers and (b) mean height of all plants occupied by *C. hypochaeridis* are greater than the diameter and height of the nearest neighbouring unoccupied plant ($N = 61$).

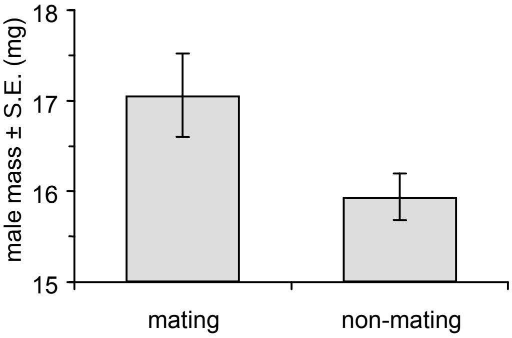

Figure 2. Figure showing that the mean mass of *C. hypochaeridis* males found mating on *L. hispidus* is greater than that of the nearest non-mating male ($N = 18$).

Field mating preferences

Mating males on *L. hispidus* flowers were significantly heavier than their nearest non-mating males (figure 2; $t_{17} = 2.47$, $P = 0.025$), where the differences in mass between the mating and the non-mating male ranged from –1.0 to +5.6 mg. Where mating and the nearest non-mating males were both found on *L. hispidus* flowers, there was no difference in either the diameter ($t_{11} = 0.34$, $P = 0.738$) or the height ($t_{11} = 0.03$, $P = 0.974$) of the flowers in which they were present.

Laboratory mating preferences

The masses of chosen and unchosen males did not differ significantly on either of the two days examined (day 1: $t_{12} = 0.930$, $P = 0.371$; day 2: $t_{10} = 0.006$, $P = 0.996$). Similarly, elytra length did not differ significantly between males on either of the days (day 1: $t_{12} = 0.928$, $P = 0.372$; day 2: $t_{10} = 0.660$, $P = 0.524$).

Discussion

Our field study found that *C. hypochaeridis* showed non-random mating with respect to male mass, where having a greater mass appeared to contribute to increasing a male's mating success. This greater success of larger or heavier males has also been recorded in other beetles [*Adalia bipunctata* L. (Kearns *et al.*, 1992; Tomlinson *et al.*, 1995); *Anoplophora malasaica* (Thomson) (Fukaya *et al.*, 2004); *Brentus anchorago* L. (Johnson, 1982); *Phorocantha semipunctata* F. (Hanks *et al.*, 1996); *Plectrodera scalator* (F.) (Goldsmith *et al.*, 1996); *Psacothea hilaris* (Pascoe) (Fukaya, 2004); *Stator limbatus* (Horn) (Savalli and Fox, 1998); *Trirhabda canadensis* (Kirby) (Brown, 1993); *Zorion guttigerum* Westwood (Wang, 2002)], although it may in some cases be driven by an interaction between mass and other traits such as coloration (Ueno *et al.*, 1998). However, we did not find the same result in our laboratory mate choice experiments, where females showed no discrimination for larger males when faced with a binary choice. This suggests that females are not actively discriminating between males of different sizes, and suggests that some other mechanism, such as competition between males, may be operating in this species.

We found that beetles were found in taller, larger flowers. Furthermore, females were observed to lay their eggs in the flowers once mating had occurred. These results suggest that females may be choosing the flower on which to lay their eggs, and mating with whichever male is present (we observed that beetles on flowers were either mating pairs, or single males). This could in turn mean that males compete for access to flowers of the preferred form, either by competing directly (as in *Zorion guttigerum* Westwood, where males fight for feeding and mating territory and larger males have a greater chance of winning fights – Wang, 2002) or indirectly (as in *Phoracantha semipunctata* (F.), where larger males have an advantageously wider antennal spread when sweep-searching an area for mates – Hanks *et al.*, 1996). However, our evidence does not support this: we observed no relationship between the size or height of *Leontodon* flowers and the mass of successful and unsuccessful males in the field. Therefore, it is unlikely that males compete directly for access to flowers based upon their height or diameter.

Overall, these results suggest that size-dependent mate choice in *C. hypochaeridis* depends upon more than a single size-dependent cue, or competition between males for a preferred site. It should be noted that our experimental procedure did not stop the beetles from mating: we found that females readily copulated (and laid eggs) in the laboratory, suggesting that the laboratory conditions themselves did not have an adverse effect upon mating behaviour. It could be the case that female *C. hypochaeridis* may have used a cue to assess size that was confounded by the experimental protocol. For example, female Silphid burying beetles *Nicrophorus orbicollis* Say are able to discriminate for larger males on the basis of pheromone cues alone (Beeler *et al.*, 2002). However, other species of beetle that use pheromones for mate choice do not show a correlation between male body size and female choice (such as in the Bostrichid *Prostephanus truncatus* (Horn) – Birkinshaw and Smith, 2001). In the present study, it is possible that females are choosing to mate on the basis of large-male pheromones being present, but are confounded as to which male is the larger by the choice arena artificially constraining how these pheromones are spread and received. However, we are uncertain that pheromones will be an important cue in *C. hypochaeridis*: the ecology of burying beetles dictates that pheromones will be much more localised to an individual than in *C. hypochaeridis* (where individuals are found in much more open areas than *N. orbicollis*), and therefore will probably be able to convey much more information about the individual. Furthermore, little is known about the pheromones produced by male Chrysomelid beetles (Dickens *et al.*, 2002; Rao *et al.* 2003).

Mate choice in the laboratory occurred either randomly, or through females responding to some unmeasured male characteristic, such as persistence (Parri *et al.*, 1998). Multiple mating is a common reproductive strategy in the Chrysomelidae (Dickinson, 1997). Our observations of the stereotypical male behaviour of tapping and stroking the female during mating suggests that 'copulatory courtship' may be occurring, with cryptic female choice for suitable sperm occurring before egg-laying (Eberhard, 1996, 1997). This could suggest that cryptic female choice is occurring in *C. hypochaeridis*, and so it is unimportant which of a pair of males a female mates with first in the laboratory, if she is later able to pick and choose which should father her offspring. Our results may also have been confounded by capturing females with differing degrees of mating experience (note that all females were mating when they were captured, and so our sample did not include virgin females). An individual's choice of mates can change in response to number of earlier matings an individual has experienced (Bateman *et al.*, 2001), and it has been shown in *Drosophila melanogaster* Meigen that female preference can change if the female has prior experience of the range of males available (Dukas, 2005). Therefore, mating experience should perhaps be taken into account when examining mate choices. Similarly, immobilising the individuals for sexing involved exposing them to CO_2, which could have an effect upon subsequent behaviour (Hoback and Stanley, 2001): further work using individuals that were not exposed to hypoxic conditions would allow us to judge whether CO_2 had an effect upon long-term behaviour, but the continued lack of a trend on the first and second day of the laboratory experiment suggests that there were no short-term effects.

On the other hand, if mating is not random, then why should there be a lack of preference in our experiment? It is conceivable that the lack of a suitable mating-platform in the laboratory may have lead to random mating. Although we established a general preference for flower size and height, we did not assess other characteristics which may be more diagnostic of a 'good mating flower' *e.g.* flower age (younger flowers may be more nutritious), pollen content, or aspect and visibility to seed-predators. Also, the method we used to determine beetle flower preference did not take the sex of the beetle into account, and it may be that females show differing selective strategies to males. Ideally, further studies should be conducted in the field to establish differences in female and male preference, as well as the outcomes of possible male competition – these should be tractable with carefully designed field experiments.

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