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Selection on morphological traits and fluctuating asymmetry by a fungal

parasite in the yellow dung fly

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Running title Selection by a fungal parasite in dung flies

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1   **Abstract** (259 words)

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3   Evidence for selective disadvantages of large body size remains scarce in general. Previous

4   phenomenological studies of the yellow dung fly *Scathophaga stercoraria* have demonstrated

5   strong positive sexual and fecundity selection on male and female size. Nevertheless, the

6   body size of flies from a Swiss study population has declined by almost 10% from 1993 to

7   2009. Given substantial heritability of body size, this negative evolutionary response of a trait

8   that is measurably positively selected suggests important selective factors being missed. We

9   took advantage of a periodic epidemic outbreak of the fungus *Entomophthora scatophagae* to

10 assess selection exerted by this fatal parasite. Fungal infection varied over the season from ca.

11 50% in the cooler and more humid spring and autumn to almost 0% in summer. The

12 probability of dying from fungal infection increased with adult body size. Females never laid

13 any eggs after infection, so there was no fungus effect on female fecundity beyond its impact

14 on mortality. Large males showed the typical mating advantage in the field, but this pattern of

15 positive sexual selection was nullified by fungal infection. Mean fluctuating asymmetry of

16 paired appendages (legs, wings) did not affect the viability, fecundity or mating success of

17 yellow dung flies in the field. Our study demonstrates rare size-selective parasite-mediated

18 disadvantages of large adult body size in the field. Reduced ability to combat parasites such

19 as *Entomophthora* may be an immunity cost of large body size in dung flies, although the

20 hypothesized trade-off between fluctuating asymmetry, a presumed indicator of

21 developmental instability and environmental stress, and immunocompetence was not found

22 here.

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1 Introduction

2 Systematic quantification of selection has become one of the hallmarks of modern biological

3 research so as to acquire a thorough understanding of the process of natural selection and its

4 evolutionary consequences. Standardized measures of selection have been available for some

5 time (Arnold & Wade, 1984a,b; Lande & Arnold, 1983; Brodie *et al*., 1995) and have been

6 applied to many species and situations to foster several comparative (meta-)analyses, which

7 greatly enhanced our understanding of the action of natural selection in the wild (e.g. Endler,

8 1986; Kingsolver et al., 2001; Kingsolver & Pfennig, 2004; Cox & Calsbeek, 2009).

9 Phenomenological investigations of selection also are the field method of choice to

10 understand the evolution and population biology of single species and populations in an

11 integrative way, and to test hypotheses about the evolution of particular traits and patterns

12 (e.g. sexual size dimorphism: Blanckenhorn, 2007).

13   The widespread yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae) is a

14 classic model species for studies of natural, particularly sexual selection (Parker, 1979;

15 Borgia, 1982; Sigurjónsdóttir & Snorrason, 1995). Phenomenological field studies have

16 established temporally variable but on average very strong mating advantages of large males,

17 as well as fecundity advantages of large females (Jann *et al*., 2000; Blanckenhorn et al., 2003;

18 cf. Honek, 1993). Strong sexual selection on male body size likely is the main driver of the

19 untypical male-biased sexual size dimorphism in *S. stercoraria* (Fairbairn, 1997; Kraushaar &

20 Blanckenhorn, 2002; Blanckenhorn, 2007, 2009). Nevertheless, the body size of flies from

21 our field population in Switzerland has declined by almost 10% over a 15-year period from

22 1993 – 2009 (Blanckenhorn, 2015). Given generally substantial heritability of body size also

23 in this species (Mousseau & Roff, 1987; Blanckenhorn, 2000), this negative evolutionary

24 response of a trait that is measurably strongly positively selected suggests that we are missing

25 important selective factors or episodes shaping the body size of yellow dung flies (Merilä et

1   al., 2001; Blanckenhorn, 2015; Gotanda et al., 2015). In general, and also for the yellow dung

2   fly, evidence for selective disadvantages of large body size remains scarce (Blanckenhorn,

3   2000, 2007).

4   One aspect not well studied in yellow dung flies is size-dependent survival in nature. This

5   is generally the case for small-bodied invertebrates, for which longitudinal field studies are

6   essentially impossible because individuals cannot be easily marked and followed in nature, as

7   is the case for larger vertebrates (Merilä & Hendry, 2014, Schilthuizen & Kellermann, 2014;

8   Stoks et al., 2014; Blanckenhorn, 2015). At the same time, laboratory longevity estimates

9   (e.g. Blanckenhorn, 1997; Reim et al., 2006; Blanckenhorn et al., 2007) generally do not well

10   reflect field mortality. We do have multiple field estimates of larval survivorship at various

11   conditions suggesting some counter-selection against large body size via the necessarily

12   longer development time (summarized in Blanckenhorn, 2007). However, sex- and size-

13   specific adult survivorship in the field was so far estimated only indirectly by Burkhard et al.

14   (2002) using age-grading by wing injuries. We herewith add a study of natural selection on

15   morphological traits by the fatal fungal parasite *Entomophthora spp*.

16   The parasitic fungus *Entomophthora scatophagae* regularly infects yellow dung flies in

17   Europe and North America (Hammer, 1941; Steinkraus & Kramer, 1988; Maitland, 1994;

18   Steenberg et al., 2001). Primarily at humid conditions infections can be epidemic (pers. obs.),

19   in which case infected dead flies can be found prominently exposed near cow pastures on

20   flowers, long grass or fences in a characteristic posture presumably effectively disseminating

21   fungal spores and/or attracting other flies (Maitland, 1994; Møller, 1993). Spore transmission

22   likely also occurs via physical contact, e.g. during copulation (Møller, 1993). The fungus is

23   fierce and effective at infecting and killing insects within hours or days and can be

24   manipulated, such that related, often species-specific such fungi are being employed for

25   biological control of insect pests (e.g. Steenberg et al., 2001; Nielsen & Hayek, 2006). We

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1   took advantage of a fungus epidemic at our field population near Zürich, Switzerland, in

2   2002.

3   I here assessed viability, fecundity and sexual selection on morphology and fluctuating

4   asymmetry. Morphological traits reflecting body size are often assessed in selection studies

5   (Kingsolver et al., 2001; Kingsolver & Pfennig, 2004; Blanckenhorn, 2007). Body size is one

6   of the most important quantitative traits of an organism, as it strongly affects most

7   physiological and fitness traits (Calder, 1984; Schmidt-Nielsen, 1984; Roff, 1992) and

8   exhibits several prominent evolutionary patterns in many organisms (Rensch, 1950; Fairbairn

9   1997; Blanckenhorn, 2000; Blanckenhorn & Demont, 2004; Kingsolver & Pfennig, 2004).

10   Depending on the taxon, diverse traits are typically used as surrogates of body size, which are

11   usually highly correlated (i.e. integrated) within individuals due to pleiotropy, epistasis or

12   gene linkage. Nevertheless, for functional reasons selection on various body parts may differ

13   (e.g. Preziosi & Fairbairn, 2000), producing responses in correlated traits and thus generally

14   prompting a multivariate approach (Lande & Arnold, 1983). I focused on paired appendages

15   (legs, wings), so I could also assess fluctuating asymmetry (FA; Palmer & Strobeck, 1986).

16   Small and random deviations from the a priori perfect symmetry in bilaterally symmetric

17   organisms, i.e. FA, are presumed to reflect heritable developmental instability, such that

18   individuals with good genes and/or living in good conditions can produce more symmetric

19   bodies in the face of environmental stress, ultimately augmenting their fitness. Symmetric

20   individuals consequently should have greater survival prospects (viability selection), and

21   should be more successful at acquiring mates (sexual selection: Møller & Swaddle, 1997).

22   However, especially the latter notion, and the evidence, remain controversial (Møller &

23   Thornhill, 1997; Palmer, 2000; Polak, 2003; Klingenberg, 2003; Van Dongen, 2006; Knierim

24   et al., 2007). In yellow dung flies, Liggett et al. (1993) and Swaddle (1997) found a negative

25   relationship between FA and mating and foraging success, respectively, while Floate &

1   Coughlin (2010) found no evidence for FA being a useful biomarker of environmental stress

2   exerted by toxic livestock medications (ivermectin). Our own previous studies of this species

3   found that FA is not heritable (Blanckenhorn & Hosken, 2003), that it does not increase with

4   inbreeding (or homozygosity: Hosken et al., 2000), and that FA does not affect male mating

5   success in the field, while nevertheless being negatively related to energy reserves

6   (Blanckenhorn et al., 2003). Of central relevance here is the postulated link between FA and

7   immunocompetence, such that more symmetric, but likely also larger individuals are expected

8   to better fend off internal parasites such as *Entomophthora* (see e.g. Rantala et al., 2000,

9   2004, 2007 and Yourth et al., 2002, for various insect species).

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11   **Material and Methods**

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13   **Study species**

14   Yellow dung flies occur throughout the northern hemisphere and are particularly common

15   around cow pastures in central Europe. The species prefers cooler climates. In lowland central

16   Europe, each year has a spring (March – June) and an autumn season (September –

17   November), while during the hot midsummer (July and August) the flies largely disappear

18   from the pastures due to their heat sensitivity (Blanckenhorn, 2009).

19   Adult *S. stercoraria* are sit-and-wait predators of small flying insects, from which they

20   extract protein to produce sperm and eggs (Foster, 1967). Females spend most of their time

21   foraging for nectar and prey in the vegetation surrounding pastures. About once a week they

22   visit fresh cattle (or other) dung to deposit a clutch of eggs. Larvae feed on and develop in the

23   dung. Multiple males typically wait at the dung pat to mate with incoming females.

24   Copulation usually takes place in the surrounding grass or on the dung pat; during the ensuing

25   oviposition the male guards the female against other competitors (Parker, 1970). Competition

26   among males for females is very strong as the operational sex ratio is highly male biased

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1   (Jann et al., 2000). Larvae face unpredictable spatio-temporal variation in local temperatures,

2   dung (i.e. food) quality and quantity, intra- and inter-specific competition, and dung drying,

3   all factors that ultimately largely determine their phenotypic adult body size. Towards the end

4   of the season the flies have to reach the overwintering pupal stage before the first winter frost

5   (Blanckenhorn, 2009).

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7   **Fly sampling**

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9   I sampled our population in Fehraltorf near Zurich (N47º52', E8º44') roughly once a month

10   between April and November 2002 (8 seasonal samples; total of $N = 541$ flies). Each time I

11   sampled one randomly selected but otherwise representative fresh dung pat, collecting all

12   single and paired flies on and ca. 20 cm around the pat to bring them alive to the laboratory.

13   As virtually no unpaired females occur at a pat in the field because competition for mates is

14   so intense, the number of pairs corresponds to the number of females present, and the

15   proportion of paired males corresponds to the operational sex ratio (females / males).

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17   **Laboratory procedures**

18   Although dead flies infected with the fungus were occasionally found on and around the

19   pasture, these were too rare and haphazard to be sampled systematically. Instead, all collected

20   flies were kept alive in the laboratory in single 100 ml bottles with sugar and water for up to

21   two weeks. Infected flies would develop the fungus within few days and eventually die; non-

22   infected, recovered or resistant flies would not. Females received dung to oviposit one clutch

23   of eggs, which was counted.

24   In the end, work study students measured left and right wing length as well as fore, mid

25   and hind tibia length of each fly. Mean values for these paired traits were subsequently

26   calculated, as well as signed FA as $(L - R)$, unsigned FA as $(|L - R|)$ (both in mm) and

27   unsigned, size-corrected FA as $(|L - R|) / mean(L, R)$ in %, as suggested by Palmer &

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1   Strobeck (1986). Paired traits were measured twice blindly by the same person to estimate

2   measurement error relative to fluctuating asymmetry (Palmer & Strobeck, 1986), and to

3   calculate the repeatability of all trait measurements (Becker, 1992). All measurements were

4   taken with a binocular microscope at 16x magnification.

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6   **Statistical analysis**

7   For each monthly sample, and overall, I calculated standardized viability selection

8   differentials (= gradients) for both sexes (binary variable: dead/alive = infected/uninfected),

9   sexual selection differentials for males (binary: mated/unmated), and fecundity selection

10   gradients for females based on their clutch size, using standard methods (Lande & Arnold,

11   1983; Arnold & Wade, 1984a,b; Brodie *et al*., 1995). It turns out that almost all females that

12   developed the fungus and eventually died in the laboratory did not lay any eggs, so fecundity

13   selection coefficients only refer to healthy (uninfected) females. We calculated selection

14   coefficients for each trait separately (4 morphological and 4 asymmetry traits), and

15   additionally for the first principal component (PC) of all (mean) appendages signifying a

16   compound index of body size, separately for females and males.

17   Because the sizes of all appendages were highly positively correlated, and because FA and

18   size are mathematically related (see above), we calculated only univariate linear *(β_{uni})* and

19   corresponding non-linear *(γ_{uni})* selection coefficients. To do so, for each seasonal sample we

20   produced standardized *z*-scores for trait *x* by subtracting the sample mean from each value

21   and dividing by the standard deviation: $z_i = \left(x_i - \bar{x}\right)/SD_x$. Relative survival or male pairing

22   success was computed as absolute survival or pairing success (1 or 0) divided by the sample

23   proportion of survived flies or mated males, respectively (Brodie & Janzen, 1996). We used

24   the univariate model of relative fitness on standardized body size $w' = c + \beta_{uni}z$ to estimate the

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1   linear selection intensities, and the corresponding quadratic model $w' = c + \beta_i z + 0.5\gamma_{ani}z^2$ to

2   estimate univariate non-linear selection intensities, γ_{ani}. These linear coefficients (gradients)

3   reflect the combined effects of direct and indirect selection on body size (Endler, 1986). The

4   difference of the regression coefficients from a slope of zero (the null hypothesis of no

5   selection) was tested. For estimation of the coefficients least-squares regression was applied,

6   but for tests of significance logistic regression was used in case our measures of success were

7   binary (viability and mating success: Brodie *et al.*, 1995).

8

9   **Results**

10   Fungus prevalence varied over the season and between the sexes. Infections (as high as 50%)

11   were most common during the cooler and more humid periods at the beginning (spring) and

12   the end of the season (autumn), whereas they were rare during the hotter summer (nearly 0%);

13   females were more affected than males (significant sex by season interaction: $\chi^2 = 29.80$; $P <$

14   0.001; Fig. 1). The probability of dying by fungal infection was unaffected by mean FA ($\chi^2 =$

15   1.99; $P = 0.159$) but increased with fly body size ($\chi^2 = 12.56$; $P < 0.001$; Table 1), an effect

16   that however varied among seasonal samples ($\chi^2 = 12.55$; $P < 0.001$) but not between the

17   sexes ($\chi^2 = 0.27$; $P = 0.602$).

18   Fecundity selection (based on clutch size) on female body size was significantly positive,

19   as is typical in this species (Jann et al., 2000; Kraushaar & Blanckenhorn, 2002). The

20   intensity of fecundity selection, i.e. the slope relating relative clutch size to standardized body

21   size (PC), varied significantly but unsystematically over the season (Table 1; $F_{6,109} = 3.50$, P

22    $= 0.03$). These estimates refer only to uninfected flies because all females infected with the

23   fungus died before laying eggs, and therefore do not estimate fecundity selection exerted by

24   the fungus beyond the parasite's effect on adult mortality.

1   As usual in yellow dung flies, larger males had a mating advantage (Jann et al., 2000;

2   Kraushaar & Blanckenhorn, 2002; Blanckenhorn et al., 2003; main effect of body size (PC):

3    $\chi^2 = 14.23$; $P < 0.001$), while mean FA of all paired appendages did not affect male mating

4   success ($\chi^2 = 1.17$; $P = 0.279$; Table 1; Fig. 2). Except for one seasonal sample on 29 May, the

5   large male advantage was consistent throughout the season such that sexual selection intensity

6   did not significantly vary across the season (body size by season interaction: $\chi^2 = 0.11$; $P =$

7   0.920; Table 1; Fig. 2). Interestingly, this pattern of positive sexual selection was nullified by

8   fungal infection in those 28 (of a total of 47) infected males (of a total of 370 males, of which

9   148 had a mate) found mating in the field that later succumbed to the fungus (main effect of

10   fungal infection: $\chi^2 = 7.61$; $P = 0.006$; Fig. 3).

11   Table 1 gives directional (linear) selection coefficients, β, for the overall sample. (Seasonal

12   values are given in the appendix.) Nonlinear (quadratic) selection coefficients, γ, were mostly

13   low and not significant and are therefore not presented in Table 1. The only exception was

14   female fecundity selection on body size, for which $\gamma = 0.056 \pm 0.025$ was significantly

15   positive, signifying accelerating selection (which has been reported before: Blanckenhorn,

16   2007, 2009). Leg and wing lengths were expectedly highly correlated in both sexes (range of

17   bivariate correlations: $r = 0.887$ to 0.972), whereas FA of the legs and wings were largely

18   uncorrelated (range: $r = -0.024$ to $+0.215$). Measurement of all paired traits was generally

19   repeatable using our methods ($R = 0.83 - 0.97$), which was also true for asymmetry ($R = 0.53$

20   $- 0.61$), so that FA could be discerned from measurement error (all side by individual

21   interactions $P < 0.01$), fulfilling the criteria of proper FA assessment (Palmer & Strobeck,

22   1986; Knierim et al., 2007).

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Discussion

At our Swiss study population, the entomophagous fungal parasite *Entomophthora scatophagae*, which has been described as a specific parasite of adult yellow dung flies at several sites in Europe and North America (Hammer, 1941; Steinkraus & Kramer, 1988; Maitland, 1994; Steenberg et al., 2001), shows high and generally fatal infection rates of up to 50% during the cooler and more humid periods of the year. I here documented that this fungus exerts relatively strong and consistent negative viability selection on female and male adult body size in *S. stercoraria* (Table 1). Fungal infection further nullifies the usual large male mating advantage in sexual selection (Borgia, 1982; Jann *et al*. 2000; Blanckenhorn et al., 2003; Fig. 3), but does not affect female fecundity beyond its impact on mortality. This represents the first evidence demonstrating viability disadvantages of large yellow dung flies mediated by a parasite, which is generally rare in animals and particularly invertebrates (Blanckenhorn, 2000; Kingsolver & Pfennig, 2004; Gotanda et al., 2015). These results complement previous evidence of viability disadvantages of large flies at the juvenile stage, and lend further credence to the notion that the male-biased sexual size dimorphism of yellow dung flies is indeed at evolutionary equilibrium (Blanckenhorn, 2007).

This study is merely phenomenological, so I could not assess mechanisms. Nonetheless, I speculate that the reduced parasite resistance of large flies signifies a trade-off between body size and immunity (Rantala & Roff, 2005; Schwarzenbach &Ward, 2006; Cotter et al., 2008). Based on age grading by wing injuries, Burkhard et al. (2002) found that adult age (i.e. longevity) of yellow dung flies in the field tends to be positively related to body size. Energy reserves also scale positively with body size (Reim et al., 2006; Blanckenhorn et al., 2007) and positively influence mating success (Blanckenhorn et al., 2003). Adult longevity under most environmental circumstances, including complete starvation, can therefore generally be expected to increase with body size on physiological grounds. One possible mechanism

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1   selecting against large body size is (positively) size-selective predation and/or parasitism

2   (Blanckenhorn, 2000). However, beyond expectations of a rough positive correlation between

3   predator and prey size (Brose et al., 2006; Vucic-Pestic et al., 2010), evidence for systematic

4   size-selectivity of predators is generally weak at best, also for yellow dung flies

5   (Blanckenhorn, 2000; Blanckenhorn et al., unpublished data). Size-selective parasitism has

6   been reported in some parasitoids (McGregor & Roitberg, 2000), but otherwise few data exist

7   (Zuk & Kolluru, 1998; Blanckenhorn, 2000). Rather than invoking selective attraction of the

8   parasite according to host size, I suspect that the ability to combat the parasite is

9   compromised in larger flies due to their generally greater absolute energy demands in a

10   stressful environment (trade-off hypothesis: Rantala & Roff, 2005; Reim et al., 2006;

11   Schwarzenbach & Ward, 2006, 2007; Cotter et al., 2008). Females were infected more by the

12   parasite here (Fig. 1), probably related to their generally greater reproductive burden (i.e. the

13   cost of producing expensive eggs rather than cheap sperm; cf. Nunn et al., 2009; but see e.g.

14   Rantala et al., 2007 for opposite results). Nevertheless, the standard sex differences in

15   reproductive (energetic) costs should be somewhat offset by the male-biased sexual size

16   dimorphism of yellow dung flies, which implies relatively greater costs in producing and

17   maintaining the larger and condition-dependent male body size (Blanckenhorn, 2000, 2007),

18   and might explain why size-dependent viability selection here turned out to be steeper in

19   males (Table 1). Yellow dung fly females indeed produce higher heritable levels than males

20   of phenoloxidase (PO; Schwarzenbach et al., 2005), one of the central mediators of insect

21   immunity (Schmid-Hempel, 2005; Rolff & Reynolds, 2009; González-Santoyo &

22   CórdobaAguilar, 2012), and higher PO levels decrease adult longevity in this species,

23   demonstrating a trade-off (Schwarzenbach & Ward, 2006). However, higher PO levels did not

24   lead to greater resistance against mites or another fungus (Schwarzenbach & Ward, 2007),

25   and PO is also unrelated to body size in *S. stercoraria* (Schwarzenbach et al., 2005). Overall,

1   therefore, the evidence in favor of immunity mediating the higher mortality of large-bodied

2   dung flies here remains limited.

3   In contrast to body size, fluctuating asymmetry (FA) of legs and wings influenced none of

4   the fitness components investigated here (contrary to Liggett et al., 1993, but confirming

5   Blanckenhorn et al's, 2003, earlier sexual selection study). I had expected, based on evidence

6   in other animals (Rantala et al., 2000, 2004), that low FA would be a signal of greater

7   immunocompetence augmenting resistance against parasites, but this was not found. It is not

8   unlikely that FA is a bad indicator of developmental stability in general, as various reviews

9   have revealed no clear verdict based on the available evidence on this question, so the entire

10   concept remains controversial (Møller & Thornhill, 1997; Møller & Swaddle, 1997; Palmer,

11   2000; various articles in Polak, 2003; Van Dongen, 2006; Knierim et al., 2007). In yellow

12   dung flies, beyond Liggett et al. (1993) the evidence for a role of FA in sexual selection is

13   close to nil (Blanckenhorn et al., 2003; Blanckenhorn & Hosken, 2003; this study). What

14   remains is that FA reliably indicates at least hot temperature stress in this species (Hosken et

15   al., 2000; Blanckenhorn & Hosken, further unpublished data), even though Floate & Coughlin

16   (2010) concluded that FA is no good biomarker of toxic chemical residues in cattle dung. As

17   the survival of flies infected with the fierce entomophagous fungus *Entomophthora*

18   *scatophagae* here was related to the size (discussed above) but not the fluctuating asymmetry

19   of wings and legs, I conclude that FA is no good indicator of immunocompetence in yellow

20   dung flies either (Rantala et al., 2000, 2004, 2007; Rantala & Roff, 2005; Yourth et al., 2002;

21   Schwarzenbach &Ward, 2006; Cotter et al., 2008).

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23

1 **Table 1:** Overall intensities ($\beta \pm$ 95% CI) of female and male adult viability selection (N_f =

2 171 & N_m = 370) exerted by the fungus *Entomophthora*, female fecundity selection (clutch

3 size; N_f = 126), and male sexual selection (pairing success; N_m = 370) for one Swiss

4 population of yellow dung flies (*Scathophaga stercoraria*) over the season 2002. Significant

5 coefficients are in bold ($P < 0.05$).

6

Trait	Adult viability				Female fecundity		Male mating success	
	β_f	95% CI	β_m	95% CI	β	95% CI	β	95% CI
Hind tibia length	-0.158	0.305	**-0.306**	0.252	**0.185**	0.027	**0.259**	0.149
Mid tibia length	-0.089	0.310	**-0.328**	0.277	**0.179**	0.029	**0.228**	0.150
Fore tibia length	-0.196	0.304	-0.221	0.251	**0.174**	0.029	**0.226**	0.159
Wing length	-0.254	0.307	**-0.324**	0.248	**0.179**	0.029	**0.267**	0.147
Overall PC size	-0.099	0.292	**-0.317**	0.302	**0.184**	0.029	**0.253**	0.136
Hind tibia FA	-0.020	0.333	-0.086	0.258	-0.023	0.045	0.051	0.151
Mid tibia FA	0.204	0.231	0.003	0.219	-0.026	0.041	0.010	0.173
Fore tibia FA	-0.037	0.299	0.224	0.283	-0.024	0.045	0.040	0.180
Wing FA	-0.187	0.321	0.046	0.279	0.037	0.045	-0.041	0.168
Mean FA	0.035	0.284	0.097	0.327	-0.021	0.040	0.071	0.134

7

1   **Figure 1:** Proportion of male (filled squares) and female (open circles) flies infected by the

2   fungus *Entomophthora* over the season 2002.
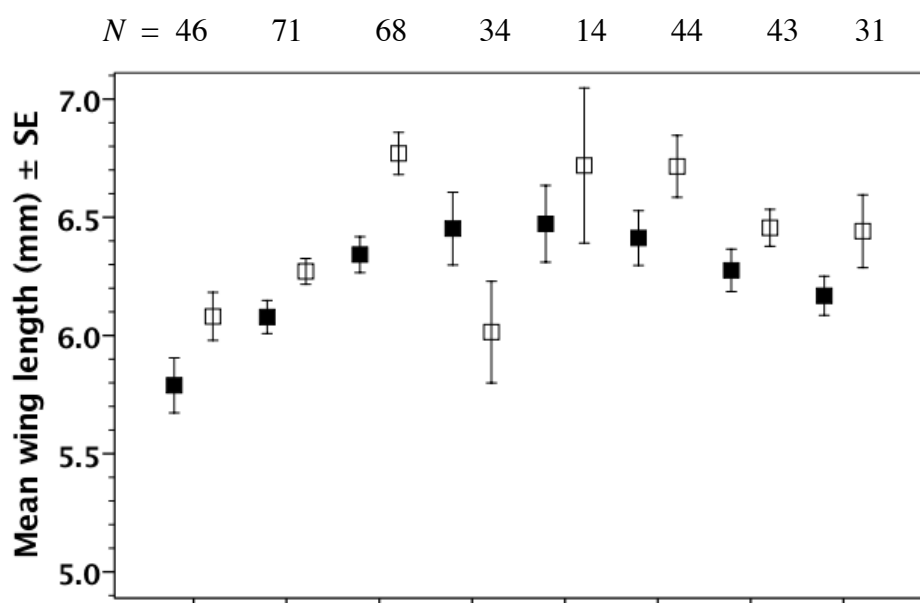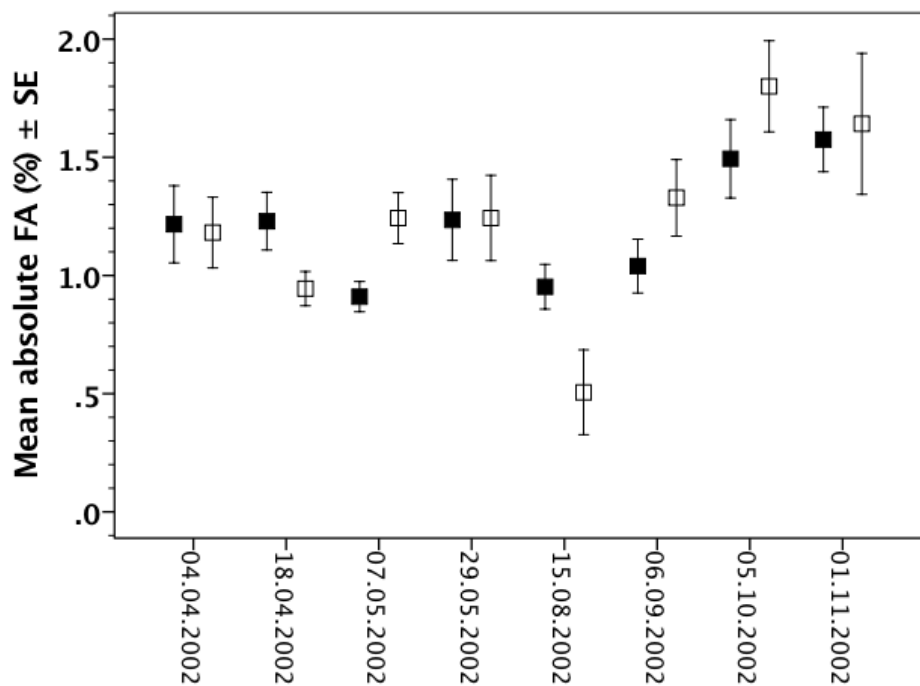
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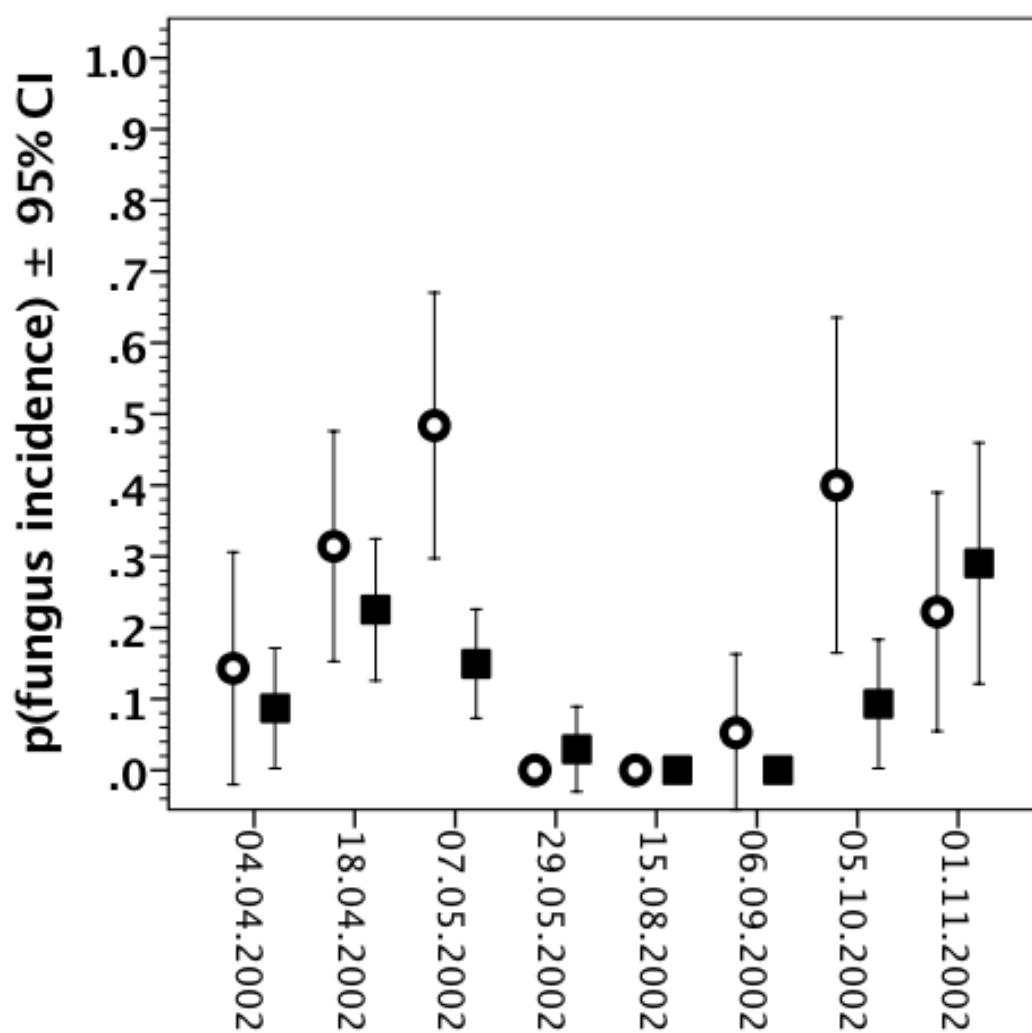
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1   **Figure 2:** Body size (top; here exemplified by wing length) and mean percentage of

2   fluctuating asymmetry (FA; bottom) of all traits for unpaired (filled squares) and paired males

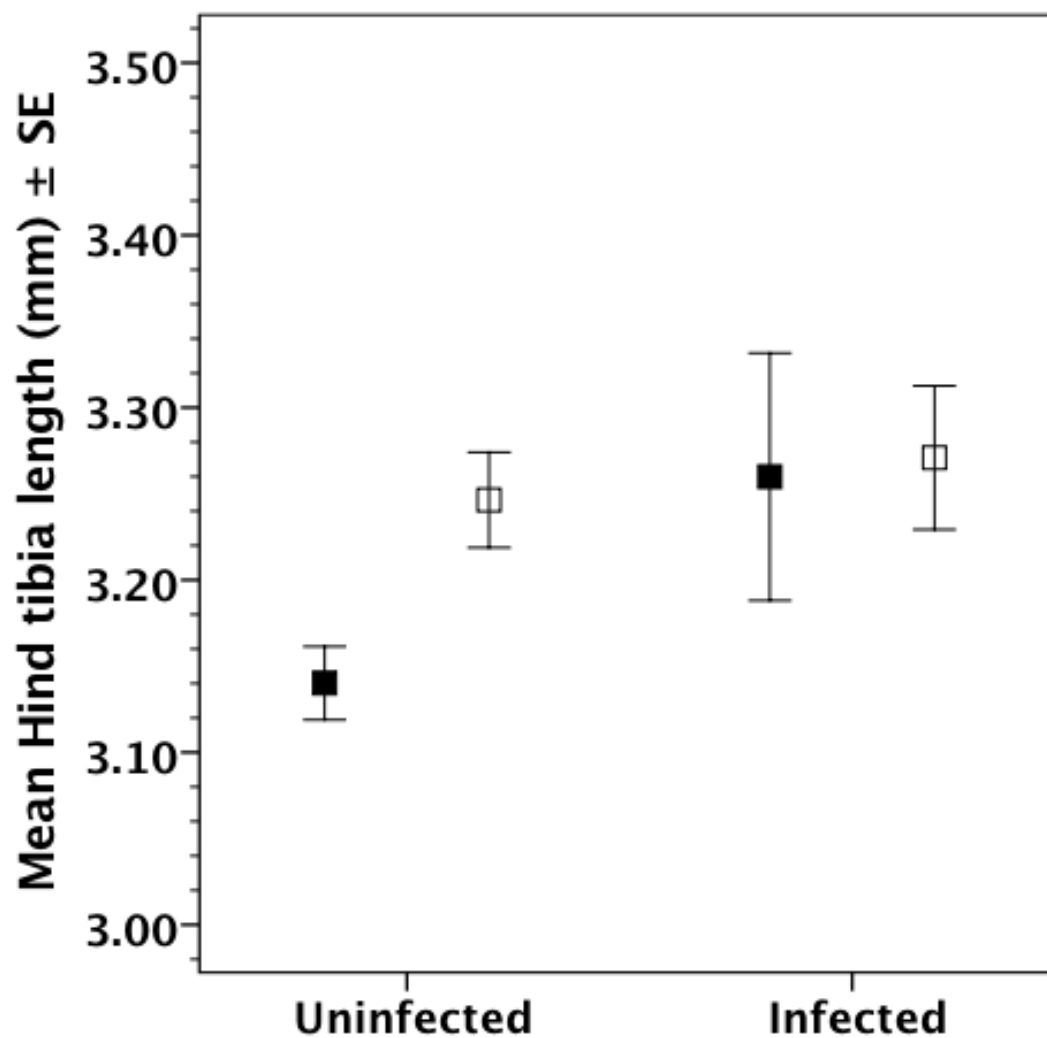3   (open squares) over the season.

4

5

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7

1 **Figure 3**: Body size (here exemplified by hind tibia length) of unpaired (filled squares) and

2 paired males (open squares) when they were infected by the fungus or not (all seasonal

3 samples combined).

4

5