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1   Artificial selection in guppies: male and female phenotypes produced by inter- and intrasexual selection

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1   **Abstract**

2   Inter- and intrasexual selection are often assumed to push phenotypes in similar directions, but this must

3   not always be the case. The current study used two artificial selection treatments in an attempt to

4   disentangle the effects of inter- and intrasexual selection in the guppy (Poecilia reticulata). The first

5   artificial treatment (INTER) was maintained by selecting males that were most appealing to prospective

6   females; in the second treatment (INTRA), only the most dominant males were allowed to reproduce.

7   Male morphology, aggression, and reproductive behaviour, as well as female choice and reproductive

8   behaviour were compared between INTRA- and INTER-treatment. After three generations of artificial

9   selection, INTER-males varied more in their orange colouration pattern than did INTRA-males. This may

10   indicate that male orange colouration is subject to negative frequency dependent selection by means of

11   female choice in the INTER-treatment. Females preferred INTER-males, regardless of their own selection

12   history. These results question the seldom tested assumption that intra- and intersexual selection

13   reinforce the same characters. The lack of direct benefits for choosing a dominant male are hypothesised

14   to be at the basis of this discrepancy, since male guppies do not defend territories and do not provide

15   resources to mates.

16

2

1   **Introduction**

2   The view that females prefer to mate with dominant males is widely accepted (Qvarnström and Forsgren,

3   1998). Yet both inter- and intrasexual selection can lead to secondary sexual characteristics. These

4   characteristics arise mostly in males, since male reproductive success is more variable and skewed

5   (Bateman, 1948). When intrasexual selection pressure is highest — e.g. polygynous mating systems —

6   traits useful in male-male combat may be targeted [such as tusks and body size in male walruses

7   (Odobenus rosmarus; Lindenfors, Tullberg and Biuw, 2002), antlers in stags (Cervidae)]. Strong

8   intersexual selection on the other hand — e.g. monogamous or polyandrous mating systems — more

9   colourful and conspicuous elaborations typically evolve [e.g. tail length in Euplectes progne (Craig,

10   1980); or complex song in Passeriformes].

11   According to the dual utility model (Berglund, Bisazza and Pilastro, 1996), male-male competition and

12   female mate choice act in a synergetic fashion and elaborate the same traits (Price and Rodd, 2006).

13   Such is the case for instance in Pimephales promela (Hudman and Gotelli, 2007); where larger males

14   win more contests over nesting sites and are also considered more attractive by females. The model

15   attempts to explain the origin of (inter)sexual secondary traits, by stating that 'armaments are ornaments'

16   and vice versa. They would function as both a 'badge-of-status' (signalling dominance) and as an

17   attraction for females (signalling fitness). This line of reasoning circumvents difficulties found in other

18   theories concerning intersexual selection; such as the need for a trait and its preference to arise quasi

19   simultaneously and become linked (Fisher, 1930), the need for a third party (i.e. parasites; Hamilton and

20   Zuk, 1982), or the need for a pre-existing female bias (Zahavi, 1975; Ryan and Rand, 1990). However,

21   the reverse situation is also plausible. Intra- and intersexual selection might act in a non-synergetic

22   fashion. The two modes of sexual selection may target different traits, as in Pavo cristatus where

23   intersexual selection operates on display behaviour and ocelli (number and density), whereas intrasexual

24   selection operates on train length (Loyau, Saint Jalme, and Sorci, 2005). Alternatively, both modes of

25   sexual selection might operate on the same traits but in opposite directions (e.g. inter- and intralocus

26   sexual conflict; Bonduriansky and Chenoweth, 2009).

27   Such non-synergetic action of inter- and intrasexual selection might occur when no direct benefits are

28   transferred by the male. When resources are lacking (or cannot be monopolised), males compete over

1   females in a direct manner. Male-male competition may confer disadvantages to females by altering the

2   number of matings, intensifying harassment and overriding female mate choice (Wong and Candolin,

3   2005; Parker, 2006 and references therein). When such disadvantages are no longer outweighed by

4   conferring resources (e.g. provision of: territory, predatory protection, a nesting site or paternal care),

5   indirect benefits (Fisherian or good-genes) may become paramount to female choice. To study the

6   interplay between inter- and intrasexual selection, guppies (P. reticulata Peters 1859) pose a suitable

7   model, since they possess a promiscuous mating system in which both dominance, and female choice

8   play part (Kodric-Brown, 1992; Bruce and White, 1995). Furthermore, the mating system is free of

9   territoriality, resource transfer or parental care (Magurran, 2005). Male guppies are also conspicuously

10   ornamented, possessing lateral colouration and large caudal fins, which as in Loyau et al. (2005) allows

11   testing whether both modes of sexual selection operate on the same traits.

12   In this study, two artificial selection treatments were maintained, mimicking the two mechanisms of

13   sexual selection. The effects of artificial sexual selection on male and female phenotypes are quantified.

14   Male phenotypes are compared, to discern how modes of sexual selection alter specific male traits.

15   Female preference and reproductive behaviour are also investigated. It is hypothesised that: a) inter- and

16   intrasexual selection may target different morphological traits. b) Increased male-male competition

17   (intrasexual selection) may increase aggression. c) Intersexually selected males will perform more

18   display behaviour, whereas intrasexually selected males will try to override female choice (force

19   copulations and harass females). d) Females may prefer males of their own selection treatment

20   [adaptation to local (social) environment]. e) Intersexual females may show higher proceptivity due to

21   lessened sexual conflict.

22

23

24   **Material and methods**

25   Species and husbandry

26   The guppies (P. reticulata) used were descendants of individuals caught in the Northern Mountain

27   Range (Trinidad; 2008) from both up and down stream sites (mixed). Thirty descendants from a

28   genetically healthy lab population were allowed to breed freely, and from their offspring, 150 individuals

1 (P; 2012) were randomly distributed over treatment groups. The initial males were photographed and

2 did not differ significantly in morphology among treatments and replicates. Two treatments are

3 maintained over four replicates. INTER-treatment males were selected in mate choice trials, with one

4 female surrounded by four males, each in their own compartment (females in central compartment; Hall,

5 Lindholm, and Brooks, 2004). Female position was scored (50 times; once every minute) when within

6 one body length and facing compartment. Trials were repeated with four independent females and

7 preference functions were made (Hall et al., 2004). For the INTRA-treatment, six males were grouped,

8 and the number of attacks (i.e. lunges, nips, and bites) initiated and endured were combined to a relative

9 score of dominance (David, 1988). Both selection treatments followed a tournament set-up, in which

10 the winners of trials were pitted against one another, until the five most preferred (INTER) and five most

11 dominant males (INTRA) were obtained (equal strength of selection). Selected males were then paired to

12 breed with two random virgin stock females (from a stock population of > 500 individuals). Young

13 virgin males were immediately separated from their sisters and grouped according to their treatment and

14 replicate (glass cuboid aquaria; 50 cm x 25 cm x 30 cm). Subsequent selection rounds were identical (F_2

15 and F_3), and individuals used here were of the third generation. While maternal effects are known to

16 exist in guppies (Eaton et al., 2015), they likely had little bearing in our standardised set-up.

17 Fish were fed twice daily (standard staple food Vipagran baby, Sera for adults; and breeding feed

18 Micron, Sera for young). Aquaria were all stored in the same room, and constantly filtered and aerated,

19 water quality was checked (pH, NO_2, NO_3) regularly, and 20% of the water was renewed weekly.

20 Temperature was kept at 25°C (thermostats; Aquarium systems Newa; Visitherm; UTX50). Lights were

21 kept at a 14/10 day-night cycle, and Fluorescent tubes ranging 150 cm length (TL-D 58W/865, Phillips)

22 overhung experiments (6500K white light; daylight). Experiments were conducted between 10:00 and

23 15:00h, and recorded using camcorders (JVC, HD Everio, GZ-U515) on stable mounts. Behavioural

24 scoring was performed using Jwatcher v1.0.

25

26 Male morphology

27 Male guppies possess orange (carotenoids and pteridines; Grether, Hudon, and Endler, 2001), black

28 (melanine; Price et al., 2008) and iridescent spots (reflecting guanine platelets; Fuji, 2000). Colouration

1   was photographed using a stereoloupe with built-in camera (LEICA, TL5000). Males were sedated

2   (dissolved clove oil in water), excess water dried off, and photographed on both sides, then returned to

3   clean water (all within two minutes, and all recovered well within ten minutes). Photographs were taken

4   under the exact same optical conditions (light and zoom) over two consecutive days. Pictures were

5   scaled (mm) and body (ichthyological standard) length, caudal fin length, body area, caudal fin area,

6   black area, orange area and iridescent area were measured (ImageJ).

7   Body and caudal fin area were taken together and summed for both left and right side measurements,

8   providing total surface area. The same was done for respective colours, and a proportion of colouration

9   was calculated ($\frac{Total\ colour\ area}{Total\ surface\ area}$) to account for size differences between individuals (Fig. S1 for

10 intercorrelations). Body length, caudal fin length, body area and caudal fin area likewise strongly

11 intercorrelate (Fig. S2). Hence, only body and caudal fin length were considered further, since both

12 caudal fin and body area showed higher measurement errors (Table S1).

13

14 Male aggression

15 To measure aggression, a mirror test was used which standardizes opponent size (Balzarini et al., 2014).

16 Focal males were placed in small white containers (28 cm x 19 cm x 9.5 cm) and water was refreshed

17 for each recording. One minute of acclimation time was granted (previous observation), then a small

18 mirror was fitted at the back end of the container. The observation period (five minutes) started when

19 the guppy approached the mirror. The number of times a male bit or lunged at its mirror image was

20 counted. Time spent; (1) swimming parallel to the mirror, (2) swimming fast alongside the mirror, (3)

21 hanging parallel next to the mirror and 4) having no interaction with the mirror image (previous and

22 trial experiments by Huyghe K.) was tracked. An index of aggression was created, with swimming

23 behaviours weighed by relative intensity ('fast swimming' * 3, 'swimming parallel' * 2, 'hanging

24 parallel' unaltered, and 'not interacting' was omitted) and summed. The index was validated by testing

25 the number of bites as a covariate in the subsequent model (ANCOVA).

26

27 Male reproductive behaviour

1   Male reproductive behaviour was investigated by placing a male together with a single random female

2   (stock; aquaria: 50 cm x 25 cm x 30 cm; back and sides covered with white paper). Small stones were

3   provided as cover and males were videotaped for ten minutes, preceded by a minute of acclimation time

4   (previous observation). Behaviours were scored as in Price and Rodd (2006; open aquarium design).

5   The number of times a male (1) nipped at the female, (2) performed a sigmoid display (male arches his

6   body horizontally into an S-shape and jerks his body; Liley, 1966) and (3) performed a gonopodial swing

7   (male flips his gonopodium back and forth; Liley, 1966) was counted. Furthermore, the time spent (1)

8   actively following (i.e. chasing) the female, (2) swimming parallel (i.e. staying in view of the female

9   with his lateral side) to the female and (3) other behaviours (i.e. no interaction) were tracked.

10

11   Female preference and reproductive behaviour

12   Virgin females (Houde, 1997; prior male exposure affects mate choice: Rosenqvist and Houde, 1997)

13   of each reproducing family unit were tested in a three-compartment design (Van Der Sluijs et al., 2013).

14   Focal females belonging to either an INTER- or INTRA-father, each underwent a preference experiment

15   by being presented to an INTER- and INTRA-male (set-up 1). Individual males were selected at random;

16   hence females were likely subjected to half or full brothers (relatedness does not bias female choice in

17   guppies; Pitcher, Rodd and Rowe, 2008). Experiments took place in two large aquaria (100 cm x 40 cm

18   x 40 cm), compartmentalised using plexi-glass barriers (without filters). Males were placed on either

19   side of the female in the central compartment. Preliminary vision was blocked by removable adjoining

20   white barriers. The female compartment was further partitioned into four zones of equal length (Z1, Z2,

21   Z3 and Z4) using red-brown tape. White paper covered all sides of the aquaria (increasing contrast and

22   visual isolation with respect to the surroundings). Fish were placed in respective compartments and

23   granted five minutes to acclimate (Cummings and Mollaghan, 2006), then white barriers were removed

24   and a ten-minute recording ensued. To avoid registering a preference for microenvironment, white

25   barriers were returned and males switched compartments. After one minute of acclimation, white

26   barriers were removed and ten more minutes of recording ensued. Association time with males (i.e. Z1

27   or Z4) was measured (valid indicator of preference: Kodric-Brown, 1985; Cummings and Mollaghan,

28   2006).

1   To investigate whether female reproductive behaviour varied relative to male phenotype, the experiment

2   was repeated with each focal female.Females were flanked by either two INTER-males (set-up 2) or two

3   INTRA-males (set-up 3). The time spent (1) following and chasing the male up against the barrier, and

4   (2) the time spent hovering up in front of the barrier facing the male were tracked. The number of (3)

5   zig zag motions and (4) up and down motions up against the barrier were counted (considered proceptive;

6   construed as actively trying to reach the male). Additionally, (5) away and return motions (Liley, 1966)

7   were counted (where the female indicates the male to follow her, which males could not do due to the

8   barriers).

9

10 Statistical analysis

11 All statistical analysis was carried out using the R 3.2.3 program. First, a correlated response among

12 replicates (A or B) within treatment (INTER or INTRA) was verified for each variable (Table S2). Only

13 variables that were not significantly different among replicates were considered further. Count data (i.e.

14 number of spots or behaviours) were analysed using generalised mixed effects models and proportional

15 data (i.e. proportion of body coloured or proportion of time spent on a behaviour) were analysed using

16 linear mixed effects models (lme4). Treatment (INTER and INTRA) was taken as fixed factor, and

17 replicates (A and B) as well as identity (female repeated measurements) were taken as nested random

18 factors. Model assumptions were validated graphically (Zuur, Ieno, and Elphick, 2010), and some

19 proportions were arcsine transformed ($\sin^{-1}(\sqrt[2]{x})$) to ensure normality (Sokal and Rohlf, 1981). Tests

20 were performed using lmerTest. Significance levels for all tests were set at $\alpha = 0.05$, and all probabilities

21 reported are two-tailed. P-values were adjusted post hoc using Holm's method (Holm, 1979) per

22 experiment.

23

24

25 **Results**

26 Male morphology

27 A correlated response among replicates could not be tested for the proportion of orange area in male

28 treatments, since model assumptions were violated. Tests for homogeneity of variances (F-tests)

1   between treatments revealed a significantly higher variance in orange area ($F_{23,44} = 8.670$, $p < 0.001$;

2   Fig. 1a) for INTER-males. The variance effect was not found between replicates, indicating a correlated

3   response to selection between replicates (orange area INTER-A vs INTER-B: $F_{7,15} = 0.835$, $p = 0.850$;

4   orange area INTRA-A vs INTRA-B: $F_{23,20} = 1.502$, $p = 0.361$; Fig. 1a and b). INTRA-males did possess

5   significantly more orange spots (INTER = 5.5 ± 1.2 spots and INTRA = 7.6 ± 1.1 spots; Table 1a and Fig.

6   2a). Other colourations and morphological traits either showed an uncorrelated response (Table S2) or

7   were not significantly different (Table 1a).

8

9   Male aggression

10   The index of aggression was validated by the ANCOVA (bites covaried significantly with the index;

11    $F_{1,64}=25.793$, $p<0.001$). However, there was no correlated response in aggression within treatments

12   (Table S2).

13

14   Male reproductive behaviour

15   On average, INTER-males performed significantly more sigmoid displays (10.6 ± 1.2 displays) at the

16   female than did INTRA-males (6.0 ± 1.2 displays; Table 1b and Fig. 2b). Other behaviours either showed

17   an uncorrelated response (Table S2) or were not significantly different between treatments (Table 1b).

18   Neither mate guarding nor copulations were observed (possibly due to stress).

19

20   Female preference and reproductive behaviour

21   Set-up 1: female preference

22   Switching male position did not have a significant effect on female behaviour nor preference ($F_{1,163} =$

23   0.551, $p = 0.459$) and was further omitted as a factor. On average, females spent a significantly longer

24   percentage of their time in the company of INTER-males (53.83 ± 8.15 %) than in the company of INTRA-

25   males (27.11 ± 11.53 %; Table 2a and Fig. 3a). Preference for INTER-males did not vary with female

26   treatment nor was there an interaction between male and female treatment (Table 2a), i.e. females did

27   not prefer males of their own selection treatment nor showed preference for opposite treatment males.

28   Due to the low sample size in this experiment (Fig. 3; bold faced numbers indicate total number of

1   unique individuals used, independent of repeated measurements), a power estimation was performed

2   (Fig. S3); e.g. showing approximately 21% statistical power for the male treatment effect.

3

4   Set-up 2 and 3: female behaviour

5   Females performed significantly more up and down motions towards INTER-males (6.6 ± 1.5) as opposed

6   towards INTRA-males (3.4 ± 1.1; Table 2b and Fig. 3b). Females showed no other significant differences

7   in behaviour towards males, nor did female treatments themselves differ significantly in their behaviours

8   (Table 2b; and uncorrelated response for the away and return motion Table S2).

9

10

11   **Discussion**

12   Male morphology

13   INTER-males had a more variable orange colouration pattern. Since INTER-males were selected by female

14   preference, this argues against directional or stabilizing selection. Most studies find that an increase in

15   orange colouration (both chroma and area) is preferred by females (Kodric-Brown, 1985; Brooks and

16   Endler, 2001). Our observation, on the other hand, seems to agree with several studies done where

17   negative frequency dependent sexual selection was found in guppies (Farr, 1977; Hughes et al., 1999;

18   Eakley and Houde, 2004). Specifically, female preference for rare male guppies has already been found

19   irrespective of familiarity and relatedness (Zajitschek and Brooks, 2008; Hampton, Hughes and Houde,

20   2009). A further consideration is whether orange area (under genetic control) or orange chroma (dietary)

21   is important (Magurran, 2005). Most studies that attribute a mating advantage to carotenoid colours

22   measure area rather than chroma. Chroma was not measured here and males might still differ in chroma

23   even under standardised laboratory conditions, should male treatments invest differentially into

24   carotenoids. Additionally, solely selecting in the male line slows down selection in autosomes and X

25   chromosomes relative to Y chromosomes (personal communication: Lindholm A., 23 October, 2015).

26   Postma et al. (2011) found that mainly orange is a Y-linked trait (sexually dimorphic traits may become

27   linked to sex chromosomes to resolve intralocus sexual conflict; Brooks and Postma, 2011; Postma et

28   al., 2011). Taken together, this would explain the prevalent effect of orange in this design.

1   Other studies have found that increased iridescence is a preferred trait (Brooks and Endler, 2001), but

2   that it is part of an alternate 'fitness peak' (Blows, Brooks, and Kraft, 2003). Black colouration, on the

3   other hand, is considered a visual signal amplifier (Brooks, 1996) that provides contrast in colouration

4   (Blows et al., 2003).

5

6   Male aggression

7   Male aggression was investigated with a possible link to dominance, yet no correlated response was

8   found within treatments which may be related to methodology. While the mirror image stimulus (MIS)

9   helps standardise measurements, it is not always consistent with live opponents (Balzarini et al., 2014:

10   e.g. due to social structures of species, behavioural repertoires and a parallel image rather than an anti-

11   parallel live opponent; Ruzzante, 1992: hormonal cues in dominance relationships). Eaton et al. (2015)

12   effectively used the MIS in guppies to test sons of mildly stressed and unstressed mothers, but validation

13   using live opponents is still lacking. Holtby (1992) emphasised that aggressiveness and dominance do

14   not correlate in all fish species, and we are inclined to argue the same for guppies. In future, tests with

15   live opponents will be carried out to elucidate effects of dominance.

16

17   Male reproductive behaviour

18   Sigmoid displays, were performed the most throughout the experiment and mostly by INTER-males,

19   agreeing with several other studies associating display rate with female interest (Farr, 1980; Bischoff,

20   Gould, and Rubenstein, 1985). Display length and intensity were not quantified, and these traits might

21   also be relevant (display quality; Houde, 1997). An increased number of nips at the female was expected

22   from INTRA-males in terms of harassment behaviour, but this was not found. These nips are often

23   directed at the female gonopore specifically. Herdman, Kelly, and Godin (2004) suggested gonopore

24   nipping is a way of assessing female reproductive state, possibly through pheromones (Crow and Liley,

25   1979; males attracted to water that previously housed reproductive females). However, gonopore nips

26   seemed to scare females (personal observation). Other behaviours did not show a correlated response,

27   nor did they differ significantly. This may be due to: 1) the use of random females (different levels of

28   receptivity among females), 2) to few episodes of selection, and 3) stress during trials (e.g. no

1 copulations were observed. Stress factors were likely the white background, high light intensity and

2 minimal cover (all done to maximise observer visibility).

3

4 Female preference and reproductive behaviour

5 Sample size and consequently statistical power were low in this experiment. However, the results of all

6 female choice set-ups showed a concurrent preference for INTER-males. This was true for both INTRA-

7 and INTER-females. This preference indicates a discrepancy in guppies between intra- and intersexual

8 selection (Qvarnström and Forsgren, 1998). Although it is not strictly verified, due to the lack of controls

9 (selection treatments may have operated similarly, but with differing intensities). Such a discrepancy

10 would be contrary to the situation in many other species where both modes of selection act in concert

11 (Coprophanaeus ensifer; Otronen, 1988; Pomatoschistus minutus; Forsgren, Kvarnemo, and Lindström,

12 1996; P. promelas; Hudman and Gotelli, 2007; Aethia cristatella; Jones and Hunter, 1999; and

13 Austrolebias charrua; Passos et al., 2013). In the aforementioned species, selection acted

14 correspondingly on body size (excluding A. cristatella; Jones and Hunter, 1999; where crests served a

15 dual utility). However, intra- and intersexual selection do not act synergistically in all species (P.

16 cristatus; Loyau et al., 2005; Libellula luctuosa; Moore, 1990; see also Qvarnström and Forsgren, 1998

17 and references therein). These species have only transient territoriality, provide no paternal investment

18 and have no resource transfer between the sexes. A lack of direct benefits can alter the interaction

19 between the two modes of sexual selection (Qvarnström and Forsgren, 1998; Wong and Candolin,

20 2005). Male guppies likewise show no territoriality, paternal care nor do they provide direct benefits to

21 females.

22

23

24 **Conclusions**

25 Higher variance of orange colouration in intersexually selected males seems to point towards negative

26 frequency dependent preference by females. Intra- and intersexual selection may not act synergistically

27 in the guppy (P. reticulata), although verification using unselected controls is needed and artificial

28 selection over more generations would further add to the study. The discrepancy in sexual selection is

1   likely rooted in the absence of territoriality, paternal investment and a resource system in their mating

2   system (lack of direct benefits for mating with dominant males).

3

11

12

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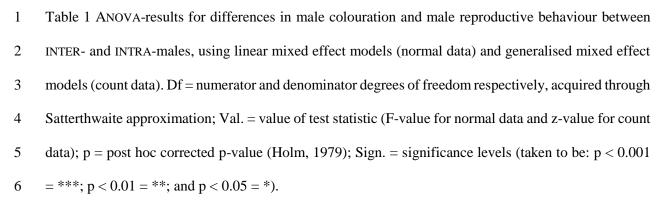
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1   Table 1 ANOVA-results for differences in male colouration and male reproductive behaviour between

2   INTER- and INTRA-males, using linear mixed effect models (normal data) and generalised mixed effect

3   models (count data). Df = numerator and denominator degrees of freedom respectively, acquired through

4   Satterthwaite approximation; Val. = value of test statistic (F-value for normal data and z-value for count

5   data); p = post hoc corrected p-value (Holm, 1979); Sign. = significance levels (taken to be: $p < 0.001$

6   = ***; $p < 0.01$ = **; and $p < 0.05$ = *).

(a) Morphology				
Trait	Df	Val.	p	Sign.
Orange spots	1,66	3.096	0.006	**
Black area[a]	1,66	0.190	0.664	
Iridescence area	1,66	3.442	0.136	
(b) Reproductive behaviour				
Sigmoid display	1,24	2.488	0.0258	*
Following	1,24	0.599	0.4465	

a = arcsine transformed data

7

8

1   Table 2 (a) ANOVA-results for differences in female association time (preference) in set-up 1 (linear

2   mixed effect model). (b) ANOVA-results for differences in female reproductive behaviour in set-up 2 and

3   3 (using linear mixed effect models for normal data and generalised mixed effect models for counts).

4   With ♀ = differences in behaviour between INTER- and INTRA-females; ♂ = differences in female

5   behaviour directed towards INTER- and INTRA-males; and ♀:♂ = interaction effect of both female and

6   male treatments. Df = numerator and denominator degrees of freedom respectively, acquired through

7   Satterthwaite approximation; Val. = value of test statistic (F-value for normal data and z-value for count

8   data); p = post hoc corrected p-value (Holm, 1979); Sign. = significance levels (taken to be:

9   p<0.001=***; p<0.01=**; and p<0.05=*).

(a) Set-up 1 (female preference)					
Behaviour	Factor	Df	Val.	p	Sign.
Association	♀	1,52	0.016	0.900	
time	♂	1,52	4.870	0.032	*
	♀:♂	1,52	1.146	0.289	
(b) Set-up 2 and 3 (female behaviour)					
Follow[a]	♀	1,12	0.225	1	
	♂	1,96	2.675	1	
	♀:♂	1,96	0.018	1	
Hover[a]	♀	1,11	0.073	1	
	♂	1,96	1.248	1	
	♀:♂	1,96	0.077	1	
Other[a]	♀	1,12	0.266	1	
	♂	1,96	0.911	1	
	♀:♂	1,96	0.014	1	
Up and down	♀	1,12	0.926	1	
motion	♂	1,96	6.152	<0.001	***
	♀:♂	1,96	1.748	1	
Zig-zag motion	♀	1,12	0.346	1	
	♂	1,96	0.935	1	
	♀:♂	1,96	0.271	1	

a = arcsine transformed data

10

11

1   Fig. 1 (a) Proportion of orange colouration with respect to total surface area for INTER- and INTRA-males,

2   showing higher variation in INTER-males and a correlated response to selection among replicates. (b)

3   Number of orange spots for INTER- and INTRA-males, likewise showing higher variance in INTER-males

4   and a correlated response to selection among replicates. Bold faced numbers denote sample size.

5

6   Fig. 2 (a) Mean number of male orange spots per treatment, with INTRA-males showing significantly

7   more orange spots (Table 1a). (b) Mean number of male sigmoid displays, with inter-males showing

8   significantly more displays (Table 1b). Error bars denote standard error and bold faced numbers denote

9   sample size.

10

11   Fig. 3 (a) Female preference indicated through association time (%) in set-up 1, where each female was

12   given the choice to interact between an INTER- and INTRA-male. Recorded twice for each female

13   (repeated measures). INTER-males were preferred significantly (Table 2a). (b) Mean number of up and

14   down motions by females directed at males in set-up 2 (INTER-males) and set—up 3 (INTRA-males),

15   recorded twice for each female (repeated measures). INTER- males elicited significantly more responses

16   (Table 2b). Error bars denote standard error and bold faced numbers denote sample size.