

Tibial coloration, fluctuating asymmetry and female choice behaviour in the damselfly *Platycypha caligata*

MICHAEL D. JENNIONS

Department of Zoology, University of the Witwatersrand, South Africa

Department of Zoology, University of Oxford

and

Smithsonian Tropical Research Institute, Republic of Panama

(Received 6 March 1997; initial acceptance 12 June 1997;
final acceptance 25 August 1997; MS. number: A7876)

Abstract. The territorial damselfly *Platycypha caligata* (Odontata: Chlorocyphidae) has a courtship behaviour where males wave the white anterior surface of all six laterally enlarged tibiae at females. I experimentally altered this white tibial surface using black paint to determine the effect on male behaviour of a 25% reduction in area, or an increase in asymmetry between the left and right side of the body. I collected behavioural data on courtship, mating and fighting for males already holding territories. Neither a reduction in the area nor an increase in asymmetry of tibial whiteness affected male mating rate, courtship rate or fighting behaviour. These manipulations also had no significant effect on the daily presence at the study site. Males whose tibial whiteness was experimentally removed also showed no decrease in mating rate or change in fighting behaviour while territorial. The complete removal of tibial whiteness did, however, lead to a significant reduction in daily presence, possibly due to a reduced ability to acquire or hold a territory. There was no relationship between natural levels of asymmetry in tibial whiteness and mating rate, courting rate or fighting behaviour for males with territories. However, the natural area of tibial whiteness was significantly positively related to both mean male mating rate and copulation duration for territorial males. This result suggests that a phenotypic correlate of area of tibial whiteness, probably body size, is sexually selected through female choice among males that already hold territories.

© 1998 The Association for the Study of Animal Behaviour

Female mate choice may increase female fitness because of direct benefits such as greater parental care; or because of indirect genetic benefits for offspring which arise due to heritable variation among males in intrinsic viability and/or attractiveness (Andersson 1994). Møller (1990) suggested that developmental stability may be a target of females' choice because it reflects the overall ability of the individual to cope with environmental and genetic stress. There is some evidence that developmental stability shows low but significant heritability (Møller & Thornhill 1997; but see Palmer & Strobeck 1997); females mating with males showing greater developmental stability should therefore produce offspring better equipped to handle environmental stress, thereby showing greater viability. Alternatively, intrinsic

female preferences for features associated with developmental stability, such as symmetry (e.g. Johnstone 1995), may result in Fisherian benefits (greater 'attractiveness') for the sons of females that choose to mate with developmentally stable males. Developmental stability is generally correlated with the ability to resist environmental stressors such as parasites (e.g. Møller 1996a) and acquire resources (e.g. Nilsson 1994; reviewed in Parson 1990). Stability, therefore, should also be correlated with resource-holding potential, fighting ability, dominance status and components of fitness that arise through male–male competition (e.g. Thornhill & Sauer 1992).

Asymmetry in traits showing fluctuating asymmetry is one measure of developmental stability. Fluctuating asymmetry occurs when small, random deviations from perfect symmetry occur in a bilaterally symmetrical trait. These subtle asymmetries arise due to developmental disruptions

Correspondence: M. D. Jennions, Smithsonian Tropical Research Institute, Unit 0948, APO AA 34002-0948, U.S.A. (email: gam128@gamboia.si.edu).

caused by genetic and environmental stress (reviewed in Palmer & Strobeck 1986). These small asymmetries will reflect aspects of quality if higher-quality individuals are more resistant to stress and develop more symmetrical traits (Møller & Pomiankowski 1993). Sexually selected traits may be particularly good at revealing developmental stability. First, their production is often associated with severe physiological stress (Møller 1996b). Second, traits under directional selection may be developmentally less stable than traits under stabilizing selection. This instability may increase phenotypic and genetic variance among males (Møller & Pomiankowski 1993) and may make it easier for females to discriminate between males on the basis of asymmetry.

Symmetry correlates with male mating success (reviewed in Watson & Thornhill 1994; Allen & Simmons 1996). Although some researchers have reported no such relationship (e.g. Markow et al. 1996; Jennions, in press), most evidence supports the claim that developmentally stable males have a mating advantage (Møller 1996a). It is less clear what mechanisms lie behind the relationship between symmetry and mating success. Females may choose males based on direct assessment of the symmetry of sexual traits (e.g. Møller 1992; Swaddle & Cuthill 1994; but see Oakes & Barnard 1994; Jennions, in press). Males could also signal fighting ability through badge or weapon symmetry and thereby raise their mating success via male–male competitive effects, but this has yet to be shown. Other studies have shown conclusively that symmetry is only a correlate of male attractiveness and not a direct target of selection by females (e.g. Thornhill 1992). Further studies have shown that asymmetrical males are less successful during male–male competition (e.g. Liggett et al. 1993; Allen & Simmons 1996), probably due to lower mechanical efficiency. Finally, trait asymmetry may have direct negative effects on functions, such as locomotion or foraging efficiency, with indirect consequences that reduce male attractiveness (Møller 1991).

In this study, I tested whether the symmetry of a secondary sexual character was directly selected through female choice, or through male assessment of symmetrical opponents as stronger fighters. To do this, I had to manipulate trait symmetry experimentally without any confounding effects on male locomotion (Balmford et al. 1993). I used black paint to manipulate the apparent size and

symmetry of sex-limited, white leg patches prominently displayed by male damselflies, *Platycypha caligata*, during courtship and male–male encounters. The behavioural context in which this coloration is displayed (Robertson 1982) and recent experimental work suggested that it functions in mate attraction (Telford et al. 1996).

Males with symmetrical patterns of coloration should have higher mating success if there is direct female choice for symmetry. They should spend less time in male–male encounters if males use symmetry to assess fighting ability. The size of a coloured wing spot is causally related to male territory-holding ability in the damselfly, *Hetaerina americana*, suggesting that males can directly assess signal size (Grether 1996b). Several studies of odonates hint at sexual selection through female choice on wing or body coloration, but methodological problems make most of these studies inconclusive (reviewed in Grether 1996a). I therefore tested whether *P. caligata* females preferred males with more white coloration. Finally, I tested whether the natural size and symmetry of white coloration could be used to predict male mating success.

METHODS

Study Species

Platycypha caligata is an unusually robust damselfly that breeds in fast-flowing, rocky streams throughout sub-Saharan Africa. It shows marked sexual dimorphism. Abdominal segments 3–10 of males are cobalt blue on the dorsal side. The males' tibiae are laterally expanded with white anterior and red posterior surfaces. Females lack expanded tibiae and are a cryptic brown colour.

Males are territorial and actively defend one or more oviposition sites of driftwood or submerged tree roots. During territorial interactions, residents fly out to meet intruders. If the intruder lands, the resident reveals the white surface of his tibiae in a 'flash' display. Usually, the two males then fly towards each other while moving their abdomens from side to side. A series of parallel and vertical flights then follow, in which males move upwards in a series of jittery hops until they are sometimes more than 6 m above the stream. During these agonistic flights, males give additional red 'flash' displays by bending the tibia upward through 180° to expose the posterior

surface. If both males descend and perch in the territory, one soon flies towards the other and performs additional white 'flash' displays until the perched male is again airborne. Encounters end when one of the males leaves the territory (Robertson 1982). In most cases the resident retains control of the territory (personal observation).

Unreceptive females are found perched on rocks along the stream. They resist male courtship attempts by raising their wings above their abdomens. When females are ready to mate, they fly along the stream. A male will fly out towards a female, then turn and head back towards his oviposition site. During this return flight, his abdomen is lowered and waggled from side to side. The female follows him and lands on the oviposition site. The male then courts her visually by extending all six tibiae below the thorax so that the white, anterior surface faces the female. The tibiae are vibrated while the male moves in a slow arc around the female, continually facing her as he does so. If the female chooses to mate, she flies up to a nearby rock or bush. The male follows and clasps the female to form a tandem pair. Within a few seconds, the female bends her abdomen under the male into the copula position. On completion of copulation, the male initiates flight and carries the female back to the oviposition site, where the pair disengage. The female then lays eggs. There is no apparent mate guarding beyond that associated with territory defence (Robertson 1982). Females also lay eggs on male-defended oviposition sites without mating with the resident male (Martens & Rehfeldt 1989; personal observation). Scores of male mating success must therefore be based on direct observation of copulation, rather than on counts of the number of females present in a territory.

Study Site

I studied *P. caligata* at Tonquani Gorge in the Magaliesberg Mountains, South Africa (25°51'S, 27°30'E) from December 1994 to February 1995. The study area was an 80-m section of rock-strewn stream, approximately 3 m wide, consisting of small, still pools and short, swift-flowing sectors. Emergent vegetation was absent and visibility was good. Since this was the only stream in this section of the gorge, any males absent from the study area were unlikely to be present at other

breeding sites. The lower and upper boundaries of the study area were a long, series of large, deep pools, unsuitable for breeding, and I saw very few marked males here.

Definitions

'Tibial whiteness' refers to the area of white marking on the anterior surface of the tibiae. 'Size of tibial whiteness' is the length of this marking. 'Total tibial whiteness size' is the sum of this length for all three pairs of legs. 'Total tibial whiteness asymmetry' is the absolute value of the difference in tibial whiteness size between the left and right side of the body (i.e. $|\text{sum of tibial whiteness on left} - \text{sum of tibial whiteness on right}|$). 'Total absolute asymmetry of tibial whiteness' is the sum of the three absolute asymmetries calculated separately for each pair of legs. The tibial whiteness runs from the distal to proximal end of each tibia and is thus closely correlated with tibial length (personal observation).

Experimental Manipulation

I captured males with a hand-held net and individually marked them on the thorax using unique combinations of small dots of Humbrol enamel paint. None of these paint colours corresponded to natural body colours. I then randomly assigned males to one of five treatments (see below) in which the size and/or symmetry of the white surface of the tibia was altered using black Humbrol enamel paint.

- (1) Reduced symmetric (RS): I painted black $\pm 25\%$ of all six tibiae ($N=61$ males).
- (2) Reduced asymmetric (RA): I painted black $\pm 50\%$ of each tibia on one side of the body ($N=61$ males, 30 left, 31 right)
- (3) Control I (Con I): I painted white 100% of all six tibiae ($N=45$ males).
- (4) Control II (Con II): I captured, handled, then released the male without painting tibiae ($N=45$ males).
- (5) Absent (Abs): I painted black 100% of all six tibiae ($N=25$ males).

Initially, I assigned 16 males each to treatments RS and RA to assess the feasibility of the marking technique on 20–23 December 1994. Then I marked 20 sets of males involving treatments RS, RA and Con I and II on 1–7 January 1995 ($N=80$ males). A set consisted of one male per treatment

Table I. Mean \pm SE morphometrics (in mm) of natural tibial whiteness for males recaptured after three or more 20-min observational samples

Character	Treatment					<i>P</i>
	Reduced asymmetric	Reduced symmetric	Control I	Control II	Removed	
<i>N</i>	14	8	12	12	10	
Mean total length per side	12.53 \pm 0.41	12.19 \pm 0.40	12.31 \pm 0.30	12.47 \pm 0.43	12.19 \pm 0.31	0.27
Hind legs	4.91 \pm 0.17	4.77 \pm 0.15	4.80 \pm 0.11	4.84 \pm 0.33	4.79 \pm 0.15	0.39
Middle legs	4.02 \pm 0.15	3.95 \pm 0.11	3.99 \pm 0.11	4.05 \pm 0.16	3.92 \pm 0.11	0.23
Front legs	3.56 \pm 0.15	3.50 \pm 0.10	3.52 \pm 0.09	3.54 \pm 0.14	3.51 \pm 0.09	0.65
Total asymmetry between sides	0.18 \pm 0.29	0.09 \pm 0.04	0.13 \pm 0.12	0.16 \pm 0.20	0.12 \pm 0.08	0.98
Total absolute asymmetry	0.26 \pm 0.29	0.21 \pm 0.18	0.17 \pm 0.12	0.21 \pm 0.25	0.26 \pm 0.15	0.56

P-values are from Kruskal-Wallis ANOVA (*df*=4).

with all males marked within 30 min of each other. Finally, I marked 25 sets of males from all five treatments on 17–31 January (*N*=125 males).

I recaptured and killed 56 males for whom I had already obtained sufficient behavioural data (see below). I then measured them in the laboratory to check whether there was any significant difference between treatments in pre-manipulation size or asymmetry of tibial whiteness. No significant differences were found (Table I). The mean \pm SE post-manipulation size of tibial whiteness per painted leg for RA males was 50.5 \pm 1.28% of the original (*N*=39 tibiae); for RS males it was 73.4 \pm 0.85% of the original (*N*=50 tibiae). These are very close to the intended values of 50 and 75%, respectively. This resulted in a total tibial whiteness asymmetry of 0.13 \pm 0.05 mm for RS males (*N*=6); and 6.26 \pm 0.09 mm for RA males (*N*=11). Mean natural total tibial whiteness asymmetry was 0.14 \pm 0.03 mm. Post-manipulation total tibial whiteness size did not differ between RS and RA males (Mann-Whitney *U*-test: *Z*=1.66, *P*=0.10). The manipulation was therefore successful because RA and RS differed in symmetry but not size. Reflectance photometry confirmed that the black paint had a flat reflectance spectra for wavelengths between 300 and 700 nm and did not reflect ultraviolet light (A. T. D. Bennett, personal communication).

Behavioural Data

I made daily censuses and noted the identity, location and time of first sighting for each male. I used these data to calculate the total number of

days a male was re-sighted. I then made detailed 20-min focal samples of individual males between 1030 and 1600 hours. I collected data only when the male was defending a territory. Territoriality is characterized by site fidelity, following other males through the site ('patrolling'), agonistic interactions (which the resident usually wins) and the presence of an oviposition site, which the male often visits and inspects by landing on it and treading the surface (Robertson 1982). This suite of behaviours is distinctive when compared to that of a wandering male. Males that are not defending an oviposition site usually move frequently, so it was difficult to obtain a 20-min sample from them. Data were discarded if the male left before the sample was completed because, in most cases, this meant that the male was not territorial.

In each focal sample, I recorded the time in flight (i.e. the time from take-off to landing) spent interacting with conspecific males. Where possible I noted the identity of opponents, allowing me to estimate the minimum number of opponents a male encountered. I also recorded the number of courtship attempts and matings. No male was sampled more than once per day. I chose which males to sample by starting at a haphazard point along the stream and selecting the first available marked, territorial male. I then moved on to the next closest male. The one caveat is that I would choose males that had been previously sampled over an unsampled male to increase the likelihood that I would obtain three samples per male.

To check the reliability of 20-min observations as predictors of general male behaviour, I

calculated intra-class correlation coefficients for males with two or more focal samples ($N=76$ males) (Zar 1984, page 323) to test whether the measured variables showed significant within-male repeatability. Fight duration was not significantly repeatable ($F_{75,135}=0.89$, $P=0.72$), but both the number of matings per sample ($F_{75,135}=1.79$, $P=0.002$) and the number of courtship attempts per sample ($F_{75,135}=1.78$, $P=0.002$) were significantly repeatable among males. I also opportunistically noted copulation duration for both marked and unmarked males.

Measuring Asymmetry

After I had observed a male for three or more samples, I attempted to capture him ($N=56$ males). I measured tibial whiteness size on each leg five times to test whether between-male variation in symmetry was significantly greater than could be accounted for by measurement error (Palmer 1994; Swaddle et al. 1994). Measurements to the nearest 0.05 mm were made under a stereomicroscope with $20\times$ magnification with a graticule-fitted eyepiece. I reduced the risk of inflating the accuracy of measurements by repositioning the tibia between successive measures so that the distal end alternated between being on the left and right side of the field of view. While measuring any given tibia, I also recorded the position of the distal and proximal end on the graticule (e.g. 3 and 73; 81 and 7). Only later were these converted into length measurements. Some damselflies lost or damaged legs during capture, and sample sizes therefore vary depending on the pair of legs in question.

Measurement of tibial whiteness asymmetry was sufficiently accurate that between-individual variation was greater than could be accounted for by measurement error (methods in Palmer 1994; front tibia: $F_{29,232}=18.5$; middle tibia: $F_{29,232}=21.0$; hind tibia: $F_{29,232}=57.0$, $P<0.0001$; $N=30$ males). To test whether data conformed to the requirements for a trait showing fluctuating asymmetry, I used t -tests to determine whether the mean values for left minus right side measures differed significantly from zero. Mean total tibial whiteness asymmetry and tibial whiteness for all three pair of legs (all unsigned) did not differ from zero (four t -tests: $t=0.27$ – 1.59 , $P=0.12$ – 0.71 , $N=41$ – 54). Therefore no pair of legs showed evidence of directional asymmetry. Although

visual inspection indicated that none of the distributions was strongly skewed or bimodal, only that for total tibial whiteness asymmetry (unsigned) did not deviate significantly from a normal distribution (Kolmogorov–Smirnov test: $P=0.18$, $N=41$). Therefore total tibial whiteness asymmetry conforms to the requirements for a trait showing fluctuating asymmetry ($|L-R|$ with a mean of zero and normally distributed).

Statistical Analysis

All pair-wise comparisons of behavioural data among males from the five treatments were made using males from sets marked over the same time period. For each male, I calculated the mean value per sample for each variable. Comparisons were planned and exhibited orthogonally. There was therefore no need to adjust the value of the type I error, and t -tests could be used to compare between two groups (Sokal & Rohlf 1995, page 231). The data in most cases, however, were non-parametrically distributed and not readily transformable. I therefore used Mann–Whitney U -tests (Siegel & Castellan 1988). The hierarchy of comparisons was: (1) between the two controls, which tested for an effect of painting tibiae; (2) between the treatments RS and RA, which tested for an effect of tibial whiteness asymmetry; (3) between RS+RA and Con I+Con II, which tested for an effect of reducing tibial whiteness size; (4) between RS+RA+Con I+Con II and tibial whiteness Abs, which tested for an effect due to the presence (75–100%) or absence of tibial whiteness.

I tested the significance of comparisons of relative frequency of dichotomous variables in 2×2 contingency tables using chi-square statistics. I used analysis of covariance (ANCOVA) to test for differences between treatments in the number of days males were re-sighted with date of first capture as the covariate. I also used ANCOVA to test for differences between treatments in the success of courtship, with the number of matings as the dependent variable and the number of failed courtship attempts as the covariates. When the interaction between covariate and treatment was not significant I excluded it from the final model (Neter et al. 1985). Unless otherwise stated, summary statistics are presented as mean \pm SE, and all tests are two-tailed.

Table II. Behavioural data (mean \pm SE) for males from reduced and control treatments

Variable	Treatment			RA	Reduced
	Reduced asymmetric	Reduced symmetric	Control	versus RS <i>P</i>	versus control <i>P</i>
<i>N</i> (males)	23	19	44		
Number of samples	2.2 \pm 0.2	2.0 \pm 0.2	2.4 \pm 0.1	0.49	0.14
Time of sampling (min)*	99 \pm 13	92 \pm 18	71 \pm 9	0.75	0.08
Time first seen each day (min)*	-7.5 \pm 12.1 (34)	-0.7 \pm 13.3 (31)	-3.3 \pm 8.7 (67)	0.86	0.85
Days seen	1.56 \pm 0.28 (32)	1.31 \pm 0.24 (36)	1.48 \pm 0.21 (63)	0.37	0.86
Mates per sample	0.05 \pm 0.03	0.18 \pm 0.10	0.15 \pm 0.04	0.41	0.14
Per cent mated	3/23	4/19	14/44	>0.25	>0.10
Time fighting per sample (s)	98.0 \pm 18.1	84.9 \pm 38.5	97.3 \pm 15.1	0.09	0.27
Number of opponents per sample	1.16 \pm 0.16	1.32 \pm 0.12	1.41 \pm 0.09	0.23	0.07
Mean number of courtships per sample	0.55 \pm 0.12	0.98 \pm 0.22	1.09 \pm 0.16	0.18	0.17

P-values are from Mann-Whitney *U*-tests. Sample sizes are given in parentheses.

*Time 0 = 1200 hours.

RESULTS

General Behaviour

I individually marked 237 males. The mean \pm SD number of days that males were re-sighted following the initial day of capture was 1.41 ± 1.57 (range=0-8 days; $N=147$). This excludes data from the 56 males that were recaptured and killed for morphological data. These 56 males were all re-sighted on at least 3 days. I obtained behavioural data from 248 20-min focal samples from 50% of the 237 males marked ($N=113$). The mean number of focal samples per male was 2.2 ± 0.1 (range=1-4). I then calculated an average value for each male for each of the measured variables. Using these averages, a 20-min sample involved 88.2 ± 10.0 s ($7.4 \pm 8.9\%$ of sampling period) in flight interacting with a minimum of 1.25 ± 0.06 males. Each male had an average of 0.91 ± 0.09 courtship attempts per sample (range=0-4), resulting in an mean of 0.14 ± 0.03 matings per 20-min sample (range=0-1.7; $N=113$ males).

Size and Asymmetry of Tibial Whiteness

I compared behavioural data from 86 males in the treatments RS, RA and Con I and II that were marked during the period 1-31 January. I first compared RS and RA males. There were no significant differences for time of sampling

($P=0.75$) or number of samples ($P=0.49$). The number of days re-sighted, time first seen each day, time spent in flight interacting with other males, minimum number of opponents per sample, number of courtship attempts per sample and number of mates per sample also did not differ between treatments (Table II). There was therefore no observable effect of increasing tibial whiteness asymmetry. There were also no differences for the any of the above variables between Con I and II males (Mann-Whitney *U*-tests: $N=21, 23$, all $P>0.10$). These results indicate that painting the tibiae did not affect male behaviour. I then performed the contrast between RS+RA (=reduced tibial whiteness) and Con I+II. There were no differences between males with reduced tibial whiteness and control males in the number of samples per male or time of day at which males were sampled. The time of day first seen, number of days re-sighted, time spent in male-male encounters, minimum number of opponents, number of courtship attempts or number of matings per sample did not differ between the two groups. When males were categorized as mated or unmated, there was also no difference in the relative frequency of mated males between the two groups ($\chi^2_1=1.91$, $P>0.10$). There was therefore no evidence that a 25% reduction in tibial whiteness influenced male behaviour.

The mean number of unsuccessful courtship attempts per male and the mean number of matings per male were positively correlated

Table III. Comparison of behaviour (mean \pm SE) between 'TW absent' and 'TW present' males

Variable	Treatment		<i>P</i> -value
	TW present	TW absent	
<i>N</i> (males)	55	14	
Number of samples per male	2.3 \pm 0.1	2.5 \pm 0.2	0.56
Time of sampling (min)*	91.1 \pm 7.8	98.0 \pm 10.1	0.73
Time first seen (min)*	2.1 \pm 5.6 (88)	-0.1 \pm 12.0 (16)	0.88
Days seen	1.97 \pm 0.21 (66)	1.06 \pm 0.35 (16)	0.021 (<i>df</i> =1,79)
Mates per sample	0.11 \pm 0.04	0.12 \pm 0.05	0.34
Per cent mating	12/55 (21.8%)	5/14 (35.7%)	>0.10
Time fighting per sample (s)	95.9 \pm 15.7	67.4 \pm 14.5	0.27
Number of opponents per sample	1.38 \pm 0.09	1.20 \pm 0.15	0.40
Mean number of courtships per sample	0.79 \pm 0.13	0.99 \pm 0.22	0.27

P-values from Mann-Whitney *U*-tests, except for 'days seen' which is from an ANCOVA (see text). Sample sizes are given in parentheses. TW: tibial whiteness.

*Time 0=1200 hours.

($r_s=0.366$, $N=86$, $P<0.01$). The relationship between courtship and mating did not differ between treatments (ANCOVA with number of unsuccessful courtships as the covariate; covariate: $F_{1,81}=9.65$, $P=0.003$; treatment: $F_{3,81}=0.72$, $P=0.54$). The interaction was not significant. Males that courted more often were more likely to mate, but the success rate of courtship attempts did not differ between treatments.

Elimination of Tibial Whiteness

I contrasted the treatments with tibial whiteness present (RS, RA, Con I and II) and the treatment 'tibial whiteness absent'. For this analysis, I used the 125 males marked over 17–31 January. There were no significant differences between the two groups in the number of samples per male or time of day at which samples were collected. There were no significant differences between the two groups in the number of matings, number of courtship attempts, number of opponents or time spent in male-male encounters per male per sample; nor in time of day first seen (Table III). When males were classed as mated or unmated, there was also no difference in the relative frequency of the two categories between treatments. ($\chi^2_1=1.14$, ns). Overall, there was no evidence that removal of tibial whiteness affected the measured behavioural variables.

Again, the number of unsuccessful courtship attempts and number of matings per male were

positively correlated ($r_s=0.46$, $P<0.01$, $N=69$). The relationship between courtship and mating did not differ between the two groups (ANCOVA with unsuccessful courtship attempts as the covariate; covariate: $F_{1,66}=26.38$, $P<0.001$; treatment: $F_{1,66}=0.113$, $P=0.74$; interaction: $F_{1,65}=1.91$, $P=0.17$). Males with and without tibial whiteness were equally successful in converting courtship attempts into matings.

The total number of days re-sighted differed significantly between the two treatments (ANCOVA with marking date as covariate, treatment: $F_{1,79}=5.574$, $P=0.021$; date: $F_{1,79}=6.339$, $P=0.014$). The interaction was non-significant. Males without tibial whiteness were seen on significantly fewer days. The proportion of males re-sighted at least once also differed between the two treatments. Males with tibial whiteness absent were less likely to be re-sighted ($\chi^2_1=3.96$, $P<0.05$; 72% versus 88%). Therefore, experimental removal of tibial whiteness reduced the male daily presence at the study site.

Behavioural Correlates of Mating

I analysed the relationship between behavioural variables and the rate of mating or courting using the mean values for males with two or more focal samples. I divided these males into those that did or did not mate while sampled (unmated: $N=51$; mated: $N=25$). The two classes of males did not differ in mean number of days seen ($P=0.70$), time

in flight interacting with other males ($P=0.24$), minimum number of opponents ($P=0.80$) or time of sampling ($P=0.42$). They differed significantly, however, in number of failed courtship attempts (Mann–Whitney U -tests: $N=51,25$, $P<0.001$): mated males courted more. The number of failed courtship attempts was weakly correlated with time of sampling ($r_s=0.216$, $P<0.10$). I therefore calculated the residuals from a regression of number of failed courtship attempts on time of sampling. These residuals also differed between mated and unmated males (Mann–Whitney U -test: $P<0.002$). Mated males had more positive values, which indicates a higher rate of courtship after correcting for time of day. The correlations between mean number of matings per sample and either the number of unsuccessful courtships ($r_s=0.414$, $P<0.001$) or the residuals from the regression of failed courtship attempts on time ($r_s=0.366$, $P<0.001$) were also significant.

Natural Size and Asymmetry of Tibial Whiteness

All pair-wise correlations between mean tibial whiteness size per pair of legs were significant ($r_s=0.70$ – 0.75 , $N=44$ – 48 , all $P<0.001$). Males with more tibial whiteness on the forelegs also had more tibial whiteness on the middle and hind legs. There were no significant correlations between pairs of legs for asymmetry (signed or absolute value; all six pair-wise correlations: $N=44$ – 48 , $P>0.09$). There also was no significant correlation between tibial whiteness size and absolute asymmetry for any pair of legs ($r_s = -0.08$ to -0.11 , $N=51$ – 54). There was a significant negative relationship between total tibial whiteness per male and total tibial whiteness asymmetry ($r_s = -0.34$, $N=41$, $P=0.03$), attributable to a single outlier with large asymmetry; when the outlier was removed from the analysis, there was no significant relationship between total tibial whiteness size and asymmetry ($r_s = -0.251$, $N=40$, $P>0.10$).

There were no significant correlations between total tibial whiteness asymmetry or total absolute tibial whiteness asymmetry and mean time of day first seen, fight duration, number of opponents, number of mates (all $N=40$) or copulation duration ($N=15$) ($r_s = -0.21$ – 0.22 , all $P>0.10$). The single exception was a significant positive correlation between total tibial whiteness asymmetry and days seen ($r_s=0.324$, $N=40$, $P<0.05$). More asymmetric males were re-sighted on more days.

Total tibial whiteness size was not correlated with the mean number of days present, time of day first seen, number of opponents or fight duration (all $N=40$), but it was significantly positively correlated with both the mean number of matings obtained ($r_s=0.376$, $N=40$, $P<0.02$) and copulation duration ($r_s=0.543$, $N=15$, $P<0.05$). Males with more tibial whiteness mated more often and longer.

DISCUSSION

Tibial Whiteness and Female Choice

Females choose among territory-holding males for mates. For male *P. caligata* holding territories, a decrease in tibial whiteness had no effect on courtship rate, mating rate or the success of courtship (matings per courtship). These results suggest that there is no directional female preference for territorial males with larger amounts of tibial whiteness. Studies of sex-limited coloration in odonates have produced mixed evidence for directional female mating preferences for greater amounts of coloration. Mating success was correlated with the width of wing pigmentation in *Libellula luctuosa* (Moore 1990); and males of the sexually dichromatic dragonfly *Plathemis lydia* had lower mating success when their abdomens were painted black (Jacobs 1955). However, in a detailed study, Grether (1996b) found no evidence for female choice of males with larger colour wing spots in *Hetaerina americana*. In *P. caligata*, there was no detectable effect of increasing tibial whiteness asymmetry on courtship rate, mating rate or the success of courtship by territorial males. These results suggest that female choice is also not based on the level of asymmetry of tibial whiteness. To date, female choice based on direct assessment of the symmetry of sexual traits has been demonstrated only in birds (Møller 1992; Swaddle & Cuthill 1994; for a negative result see Jennions, in press).

One surprising result was that complete removal of tibial whiteness did not affect courtship rate, success of courtship or mating rate by territorial males. These results suggest that female choice is not influenced by white tibial coloration, even though this trait bears all the hallmarks of a sexually selected character (sexually dimorphic and colourful). These findings also contradict Telford et al. (1996), who concluded that white

tibial markings are required for male *P. caligata* to mate. Telford et al. painted the white anterior surface of the tibiae red, and the red, posterior surface white. Thus their failure to observe matings may be because females reject males producing courtship displays with red coloration (which is normally displayed only in agonistic male–male encounters), rather than displays that lack white coloration.

Tibial Whiteness and Male Fighting Behaviour

There was no effect of manipulation of tibial whiteness size or symmetry on the proportion of time territorial males spent fighting during samples, or in the number of opponents engaged. This result, however, provides only weak evidence that tibial whiteness does not function in male fighting assessment. Samples where the focal male departed were excluded from the analysis, so any effect of the manipulations of tibial whiteness on eventual fighting success are unknown. Interestingly, the main effect of removing tibial whiteness was to reduce significantly the number of days that males were re-sighted. It is possible that the absence of tibial whiteness lowered male survivorship, or reduced foraging efficiency causing males to spend more time away from the study site feeding. Neither explanation is compelling, however, because the absence of tibial whiteness should decrease, not increase, male conspicuousness to predators; similarly, the absence of tibial whiteness should decrease any risk of early detection and evasive manoeuvring by potential prey. An alternative explanation is that the absence of tibial whiteness lowers the ability of males to acquire or hold territories. Males without territories are more likely to wander or leave the study site, which decreases their likelihood of being re-sighted. Although quantitative data were not collected, my own observations support this explanation. I found it difficult to obtain 20-min behavioural samples from males without tibial whiteness because they often moved after only a few minutes, which indicates non-territoriality. White ‘flash’ displays are given at the start of male–male encounters and to reinstate agonistic flight interactions whenever a male pauses to rest inside a disputed territory. I therefore suggest that tibial whiteness may be mainly sexually selected through functions pertaining to male–male interactions. In several taxa, especially birds, evidence

suggests that coloration (e.g. badges) is used in male fighting assessment (Andersson 1994). Grether (1996b) recently showed a causal relationship between wing spot size and a male's success at defending his territory in the damselfly, *H. americana*. This work shows that badges potentially occur in odonates. Further research on the function of tibial whiteness as a signal during fights in *P. caligata* may therefore be worthwhile.

Natural Size and Asymmetry of Tibial Whiteness

The mean amount of tibial whiteness per pair of legs showed significant correlations between all three pairs of legs. This is not surprising as the white patches extend along the full length of each tibia, and one expects males with larger hind legs to have larger middle and forelegs. In contrast to tibial whiteness size, there were no significant correlations between pairs of legs for tibial whiteness asymmetry, either signed or absolute. This finding is consistent with those of other studies of fluctuating asymmetry which also have shown little evidence for concordance among characters in their asymmetry (reviewed in Dufour & Weatherhead 1996), although Leamy (1993) showed that such correlations are more likely to be detected among developmentally related traits. Recent sexual selection studies of fluctuating asymmetry report positive correlations between male mating success and the symmetry of single traits. These results are interpreted as evidence that females select males with greater developmental stability (‘higher quality’). If asymmetry is uncorrelated between traits, however, this conclusion is sensitive to which trait is examined. The fact that most studies show that symmetry correlates with mating success (e.g. Møller 1996a) therefore suggests that researchers have fortuitously picked traits that are either unusually good indicators of overall developmental stability, or traits, like wings or weapons, whose asymmetry has a direct effect on male performance or attractiveness. In *P. caligata*, total tibial whiteness asymmetry was not correlated with the measured behavioural variables, including mating and courtship rate. Thus there is no evidence for female choice of developmentally stable males as measured by tibial whiteness symmetry.

In contrast, males with larger amounts of tibial whiteness mated more often and for a longer duration. Because males were already territorial

when observed, the correlation with mating rate suggests female choice of males with more tibial whiteness rather than any effect of male–male competition. Given that experimental manipulation of tibial whiteness did not affect mating rate, though, this outcome is unlikely to be due to direct female choice for more tibial whiteness. It is more likely that tibial whiteness is correlated with another variable that increases male attractiveness. The most obvious candidate is male size. The amount of tibial whiteness and leg size are closely related, and leg size is likely to be associated with general body size. Evidence of female choice for body size in odonates is inconclusive; most studies have confounded sexual and survival selection, and the relative importance of each therefore remains unclear (reviewed in Grether 1996b). There is evidence for a positive effect of male size on mating success in the damselfly *Megaloprepus coerulatus* (Fincke 1988), dragonfly *Orthetrum chrysostigma* (Miller 1983) and damselfly *Hetaerina americana* (Grether 1996a). The correlation between tibial whiteness and copulation duration in *P. caligata* could be due to male and/or female behaviour. Although it is usually argued that males control copulation duration, this need not always be the case (reviewed in Fincke, in press). It is therefore possible that females choose to copulate longer with larger males (i.e. those with more tibial whiteness). Given the above results it may be worthwhile in the future to look directly at the effect of male size on female choice in *P. caligata*.

For none of the three pairs of legs was there a significant negative relationship between total tibial whiteness and asymmetry. Møller & Pomiankowski (1993) have argued that condition-dependent, sexually selected traits should show a negative relationship between size and asymmetry, but it is unclear whether this relationship generally holds (Tomkins & Simmons 1996). There are also theoretical grounds on which this conclusion can be disputed (Jennions 1996). It is therefore possible that tibial whiteness is a condition-dependent trait in *P. caligata*. Indeed this is probable if larval foraging efficiency and size at eclosion determine adult body size.

Summary

Why did manipulation of tibial whiteness size or symmetry not affect female choice? In

P. caligata, female choice may be based primarily on territory quality, which should have direct fitness benefits in terms of offspring survival (Siva-Jothy et al. 1995). Moreover, males with good territories are probably in better condition, and more likely to provide direct benefits such as reduced risk of parasite transfer or indirect benefits ('good genes'). Hence, territory quality may be a better predictor of male quality than assessment of male phenotype. Several studies have shown, however, that even if choice is based on territory quality, females can still choose males based on their phenotype (e.g. Backwell & Passmore 1996). If females choose males solely on territory quality, it is also unclear why males perform an elaborate courtship display (Robertson 1982) that exceeds the requirements for species identification. Fincke (in press) has recently argued that, contrary to conventional wisdom, female choice based on male phenotype may be more important in Odonata species where males defend territories with oviposition sites. The high physiological costs of territory defence may place a premium on producing sons that are able to defend territories successfully. The finding that male *P. caligata* with more tibial whiteness have higher mating rates and longer copulation duration when territorial is consistent with female choice based on a variable correlated with tibial whiteness.

ACKNOWLEDGMENTS

I thank Pat and Maureen Backwell, Rob 'E' Brooks, David Jennions and Tom Spieker for invaluable field assistance; and Phil Bishop and Nev Passmore for logistic support. A. T. D. Bennett kindly carried out photospectrometry on the paint. Steve Telford first suggested working on *Platycypha*. Permission to work at Tonquani was generously given by the Transvaal Mountain Club; I especially thank the Secretary and Gordon Ehrens. I thank Bill Wcislo, Anders Møller, Marian Dawkins, John Christy, Pat Backwell and anonymous referees for comments on various drafts of this manuscript. Work was funded by an A.S.A.B. Research Grant, the C.B.R.G. (Wits University) and the Rhodes Trust (Oxford). STRI graciously provided facilities during the writing stage.

REFERENCES

- Allen, G. R. & Simmons, L. W. 1996. Coercive mating, fluctuating asymmetry and male mating success in the dung fly *Sepsis cynipsea*. *Anim. Behav.*, **52**, 737–741.
- Andersson, M. B. 1994. *Sexual Selection*. Princeton: Princeton University Press.
- Backwell, P. R. Y. & Passmore, N. I. 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. *Behav. Ecol. Sociobiol.*, **38**, 407–416.
- Balmford, A., Jones, I. L. & Thomas, A. 1993. On avian symmetry: evidence of natural selection for symmetric tails and wings in birds. *Proc. R. Soc. Lond. Ser. B*, **252**, 245–251.
- Dufour, K. W. & Weatherhead, P. J. 1996. Estimation of organism-wide asymmetry in red-winged blackbirds and its relation to studies of mate selection. *Proc. R. Soc. Lond. Ser. B*, **263**, 769–775.
- Fincke, O. M. 1988. Source of variation in lifetime reproductive success in a nonterritorial damselfly. In: *Reproductive Success* (Ed. by T. H. Clutton-Brock), pp. 24–43. Chicago: The University of Chicago Press.
- Fincke, O. M. In press. Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. *Biol. J. Linn. Soc.*
- Grether, G. F. 1996a. Sexual selection and survival selection on wing coloration and body size in the rubyspot damselfly *Hetaerina americana*. *Evolution*, **50**, 1939–1948.
- Grether, G. F. 1996b. Intersexual competition alone favours a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution*, **50**, 1949–1957.
- Jacobs, M. E. 1955. Studies on territorialism and sexual selection in dragonflies. *Ecology*, **36**, 566–586.
- Jennions, M. D. 1996. The allometry of fluctuating asymmetry in southern African plants: flowers and leaves. *Biol. J. Linn. Soc.*, **59**, 127–142.
- Jennions, M. D. In press. The effect of leg band asymmetry on female–male association in zebra finches. *Anim. Behav.*
- Johnstone, R. A. 1995. Female preference for symmetrical males as a by-product of selection for mate recognition. *Nature, Lond.*, **372**, 172–175.
- Leamy, L. 1993. Morphological integration of fluctuating asymmetry in the mouse mandible. *Genetica*, **89**, 139–153.
- Liggett, A. C., Harvey, I. F. & Manning, J. T. 1993. Fluctuating asymmetry in *Scatophaga stercoraria*. *Anim. Behav.*, **45**, 1041–1043.
- Markow, T. A., Bustoz, D. & Pitnick, S. 1996. Sexual selection and a secondary sexual character in two *Drosophila* species. *Anim. Behav.*, **52**, 759–766.
- Martens, A. & Rehfeldt, G. 1989. Female aggregation in *Platycypha caligata* (Odonata: Chlorocyphidae): a tactic to evade male interference during oviposition. *Anim. Behav.*, **38**, 369–374.
- Miller, P. L. 1983. The duration of copulation correlates with aspects of the mating behaviour of *Orthetrum chrysostigma* (Burmeister) (Anisoptera: Libellulidae). *Odonatologica*, **12**, 227–238.
- Møller, A. P. 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. *Anim. Behav.*, **40**, 1185–1187.
- Møller, A. P. 1991. Sexual ornament size and the cost of fluctuating asymmetry. *Proc. R. Soc. Lond. Ser. B*, **243**, 59–62.
- Møller, A. P. 1992. Female swallow preference for symmetrical male sexual ornaments. *Nature, Lond.*, **357**, 238–240.
- Møller, A. P. 1996a. Sexual selection, viability selection and developmental stability in the domestic fly *Musca domestica*. *Evolution*, **50**, 746–752.
- Møller, A. P. 1996b. Development of fluctuating asymmetry in tail feathers of the barn swallow *Hirundo rustica*. *J. evol. Biol.*, **9**, 677–694.
- Møller, A. P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. *Genetica*, **89**, 276–279.
- Møller, A. P. & Thornhill, R. 1997. A meta-analysis of the heritability of developmental stability. *J. evol. Biol.*, **10**, 1–16.
- Moore, A. J. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. *Evolution*, **44**, 315–331.
- Neter, J., Wasserman, W. & Kutner, M. H. 1985. *Applied Statistical Models: Regressions, Analysis of Variance, and Experimental Designs*. 2nd edn. Homewood, Illinois: Irwin.
- Nilsson, J. 1994. Energetic stress and the degree of fluctuating asymmetry: implications for a long-lasting, honest signal. *Evol. Ecol.*, **8**, 248–255.
- Oakes, E. J. & Barnard, P. 1994. Fluctuating asymmetry and mate choice in paradise whydahs (*Vidua paradisaea*): an experimental manipulation. *Anim. Behav.*, **48**, 937–943.
- Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer. In: *Developmental Instability: Its Origins and Evolutionary Implications* (Ed by T. A. Markow), pp. 335–364. Dordrecht, Netherlands: Kluwer.
- Palmer, A. R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *A. Rev. Ecol. Syst.*, **17**, 391–421.
- Palmer, A. R. & Strobeck, C. 1997. Fluctuating asymmetry and developmental stability: heritability of observable variation vs. heritability of inferred causes. *J. evol. Biol.*, **10**, 39–49.
- Parson, P. A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. *Biol. Rev.*, **65**, 131–145.
- Robertson, H. M. 1982. Mating behaviour and its relationship to territoriality in *Platycypha caligata* (Selys) (Odonata: Chlorocyphidae). *Behaviour*, **74**, 11–26.
- Siegel, S. & Castellan Jr, N. J. 1988. *Nonparametric Statistics for the Behavioural Sciences*. 2nd edn. New York: McGraw-Hill.
- Siva-Jothy, M. T., Gibbons, D. W. & Pain, D. 1995. Female oviposition preference and egg hatching success in the damselfly *Calopteryx splendens xanthosoma*. *Behav. Ecol. Sociobiol.*, **37**, 39–44.
- Sokal, R. R. & Rohlf, F. J. 1995. *Biometry*. New York: W. H. Freeman.

- Swaddle, J. P. & Cuthill, I. C. 1994. Female zebra finches prefer symmetric males. *Nature, Lond.*, **367**, 165–166.
- Swaddle, J. P., Witter, M. S. & Cuthill, I. C. 1994. The analysis of fluctuating asymmetry. *Anim. Behav.*, **48**, 986–989.
- Telford, S. R., Barnett, M. & Polakow, D. A. 1996. The functional significance of tibial displays in the damselfly *Platycypha caligata* (Selys) (Odonata: Chlorocyphidae). *J. Insect Behav.*, **9**, 835–839.
- Thornhill, R. 1992. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). *Behav. Ecol.*, **3**, 277–283.
- Thornhill, R. & Sauer, P. 1992. Genetic sire effects on the fighting ability of sons and daughters and mating success of sons in a scorpionfly. *Anim. Behav.*, **43**, 255–264.
- Tomkins, J. L. & Simmons, L. W. 1996. Dimorphism and fluctuating asymmetry in the forceps of male earwigs. *J. evol. Biol.*, **9**, 753–770.
- Watson, P. J. & Thornhill, R. 1994. Fluctuating asymmetry and sexual selection. *Trends Ecol. Evol.*, **9**, 21–25.
- Zar, J. A. 1984. *Biostatistical Analysis*. 2nd edn. New York: Prentice-Hall.