An analysis of multivariate selection in a non-territorial damselfly (Odonata: Coenagrionidae)

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Summary. The relationship between fitness and phenotypic traits (body, thorax and wing length, head width and date of emergence) was studied in a sample of 187 males and 113 females of the damselfly *Ischnura graellsii*, by means of a multivariate regression analysis of selection. Male fitness was estimated as lifetime mating success divided into three multiplicative episodes: lifespan, visits/lifespan and matings/visit (mating efficiency). In females, reproductive success was estimated from the lifetime number of ovipositions divided into lifespan, visits/lifespan and ovipositions/visit. Results indicated the absence of directional selection but highly significant nonlinear selection was observed in both sexes in respect to the date of emergence and body proportions. These results suggest that selection acts simultaneously on the multivariate phenotype and several traits should therefore be included in the selection analysis.

Key words: multivariate selection, phenotypic selection, body size, date of emergence, *Ischnura graellsii*, Odonata.

Resumen. *Análisis de la selección multivariante en una libélula no territorial (Odonata: Coenagrionidae).* En este trabajo se estudia la relación entre eficacia biológica y variables fenotípicas en una muestra de 187 machos y 113 hembras de la libélula *Ischnura graellsii*, mediante métodos multivariantes de regresión para el análisis de la selección. Las variables analizadas fueron longitud corporal, anchura cefálica, longitud del tórax, longitud del ala, y fecha de emergencia, estimada a partir de la edad en el momento del marcaje. La eficacia biológica de los machos se estimó como el éxito copulativo a lo largo de la vida (ECV), dividido en tres componentes multiplicativos: longevidad, visitas/longevidad y cópulas/visita (eficiencia en el apareamiento). En las hembras se usó el número de oviposiciones a lo largo de la vida (EOV) como una estima de su éxito reproductivo. EOV se dividió en longevidad, visitas/longevidad y oviposiciones/visita. Los resultados indican la ausencia de selección direccional significativa pero la presencia de selección no direccional muy significativa en ambos sexos en la fecha de emergencia y las proporciones corporales. Estos resultados sugieren que la selección está actuando simultáneamente en el fenotipo multivariante, y por lo tanto se deberían incluir varios caracteres en los análisis de la selección.

Introduction

Darwinian distinction between natural and sexual selection is based on the fact that sexual selection derives from the existence of variance in mating success, while natural selection is related to other fitness components (Darwin, 1871; Wade & Arnold, 1980; Arnold, 1983; Arnold & Wade, 1984a). Nevertheless, the existence of variance in mating success does not necessarily determine sexual selection, if all variation is explained by random factors (Stoks, 2000). If we obtain data on lifetime mating success (LMS) from a set of individuals in a natural population and we partition it into multiplicative episodes, we can then estimate the potential for selection on these components (Arnold & Wade, 1984b). Knowledge of heredity is essential for a complete evolutionary analysis; nevertheless, it is possible to measure the effect of selection on phenotypic characters, even in the absence of genetic data (Arnold & Wade, 1984b; Grafen, 1988), because most traits are heritable, and hence, likely to be able to respond to selection. It is also possible to separate components due to natural and sexual selection, which is very important because the two processes can act in opposite ways on the same character (Darwin, 1871).

In this paper I report the study of lifetime mating success (LMS) and selection on phenotypic characters in males and females of the damselfly *Ischnura graellsii* (Rambur). Males of this species are non-territorial and are involved in scramble competition searching for mates (Cordero, 1989). Females lay eggs alone, usually before the start of mating activity in the afternoon (Cordero, 1989; Cordero, 1992a). The existence of sperm removal and displacement (Cordero & Miller, 1992) and long cop-ulations (a method of “in-copula guarding”, Cordero, 1990) suggests that there has been intense sexual selection on males in the past. The aim of this

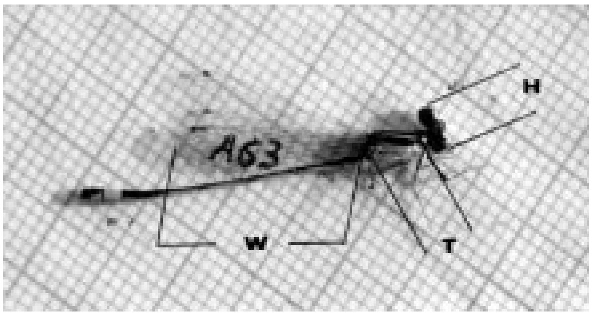

Figure 1. The phenotype of male A63, the most successful male included in the sample (4 copulations). From photographs such as this I measured wing length (W), thorax length (T) and head width (H). Scale is in mm.

study was to estimate the intensity of selection on a set of phenotypic variables, several of which are shown to be under non-linear sexual selection in this population.

Methods

Field methods and study sites.

Field work was carried out in a man-made pond during August and September 1990, at O Rosal (Pontevedra province, NW Spain). Adults were individually marked by writing a number on their wings with an indelible pen, measured (total body length, from head to tip of the abdomen to the nearest 0.1 mm) and aged (from thorax coloration, Cordero, 1987), and their reproductive status was recorded at each sighting. Individuals included in this study were marked between 11 and 23 August, although sampling continued until 8 September, in order to follow marked animals throughout their lives (9.8±0.2 h of observation per day). Walking slowly along the shore, an attempt was made to record all ovipositions and identification codes of females seen ovipositing (Cordero, 1992a; Cordero, 1995; Cordero & Egido, 1998).

Mating success and selection.

Lifetime mating success (LMS) of males was used as an estimate of reproductive success. This is appropriate because males are able to remove most of the sperm from females, resulting in virtually complete last male precedence in the first clutch, although sperm mixing is evident in subsequent clutches (Cordero & Miller, 1992). Nevertheless, this estimate ignores the variation in fertilizations/mating and female fecundity (Fincke, 1986). In the laboratory, female fecundity is mainly affected by female longevity and size (Cordero, 1991). This variation is probably of minor importance in male reproductive success because (1) most females will not reach their potential fecundity in the field due to short lifespan and (2) mating is not assortative by size (Cordero, 1995). Furthermore, some females could oviposit without re-mating (Cordero & Egido, 1998), resulting in great differences in the number of eggs that males fertilize depending on the number of subsequent matings by their mates. LMS was divided into three multiplicative episodes: lifespan, visits/lifespan and matings/visit (mating efficiency). Lifespan was estimated as: age at marking (from thoracic coloration) + observed lifespan (days between marking and last recapture) - 5 (days of maturation period). This is therefore an estimate of the mature lifespan of each individual. Visits are the number of days a male was observed during its mature lifespan.

In females, I used lifetime number of ovipositions (LNO) as an estimate of reproductive success, because females can fertilize their lifetime egg production after just one mating (Cordero, 1990). Females seen ovipositing more than once on the same day were nevertheless assigned only one oviposition that day, because I could not record the total duration of oviposition. Therefore, the number of ovipositions equals the number of days of oviposition. Number of ovipositions is positively related to lifetime egg production in the laboratory, but female fecundity is better predicted by longevity and body size (Cordero, 1991). As in males, this estimate was divided into three multiplicative episodes: lifespan (defined as for males), visits/lifespan and ovipositions/visit.

The selection of phenotypic characters was analyzed in 187 males and 113 females whose phenotypic characters were all known. I examined the selection on the date of emergence (estimated from age at marking), total body length (as measured in the field),

Table 1. Estimates of standardized directional selection gradients (ß) and standard errors (in parentheses) calculated from a linear regression model, $w_k = a + \sum ß_i Z_i$, where a=0 because phenotypic characters were standardized to mean=0 and variance=1.

		Directional selection gradients ($ß_i$)				
	Fitness component	body length	thorax length	wing length	head width	date
Males	lifespan	0.0787	-0.0724	0.0521	-0.0091	-0.1687[a]
	visits/lifespan	-0.0623	0.0227	-0.0290	0.0365	0.0898
	matings/visits	0.0475	-0.0300	0.1397	0.1395	0.0902
	LMS	0.0315	-0.1841	0.3936	0.1020	-0.0204
Females	lifespan	0.1075	-0.0872	-0.0655	0.0611	-0.2002
	visits/lifespan	-0.0086	0.0920	0.0372	-0.1149	0.0870
	ovipositions/visits	-0.3341	0.1396	0.1856	0.1338	-0.1523
	LNO	-0.0789	0.1715	0.0709	0.1039	-0.2066

[a] $p<0.1$

Table 2. Estimates of standardized non-linear and correlational selection gradients (γ_{ii} and γ_{ij}) and standard errors (in parentheses) obtained from the quadratic regression model $w_k = a + \sum ß_i Z_i + \sum \gamma_{ii} Z_i^2 + \sum \gamma_{ii} Z_i Z_j$; where a=0 because phenotypic characters were standardized to mean=0 and variance=1.

	Fitness	Non-linear selection gradient (γ_{ii})					Correlational selection gradient (γ_{ij})									
	component	body (B)	thorax (T)	wing (W)	head (H)	date (D)	B x T	B x W	B x H	B x D	T x W	T x H	T x D	W x H	W x D	H x D
Males	lifespan	0.5956**	0.2862**	0.6058***	0.1691	0.3803***	-0.2374	-1.0609***	-0.1214	0.1601	-0.1422	-0.1155	0.0800	0.1882	0.0436	-0.2628**
	visits/lifespan	0.7771***	0.2902***	0.4313***	0.3175***	0.3533***	-0.3166	-0.8705***	-0.4040	-0.2216	-0.0028	-0.1250	0.0766	0.0144	0.1901	0.0854
	matings/visits	0.3543	0.3195	0.9871***	0.1933	0.4535***	-0.4397	-0.9530	0.0633	-0.4669	-0.2745	0.2025	0.7611***	-0.3399	-0.4181	0.1704
	LMS	0.3936	0.4106*	0.7155*	0.2440	0.4438***	-0.4053	-0.6741	-0.2804	-0.3737	-0.4776	0.2774	0.4815*	-0.0942	0.1290	-0.0301
Females	lifespan	-0.0174	0.2772	0.1367*	0.6640***	0.2427***	-0.2289	0.7274	-0.5894*	-0.3372	-0.0922	-0.0173	0.1754	-0.5027	0.2351	-0.0220
	visits/lifespan	-0.2056	0.3484	0.1214	0.6112***	0.2483**	-0.1605	1.2752**	-0.6767*	-0.1927	-0.7121*	0.1573	0.0366	-0.3869	0.4546*	0.0353
	ovipositions/visits	-0.1294	0.2399	0.1785	0.5648*	0.2431*	-0.3589	1.3518*	-0.7535	-0.2269	-0.3449	0.4177	-0.0059	-0.8447	0.1686	-0.1149
	LNO	-0.5861	-0.1913	-0.0842	0.3254	-0.0248	0.4854	1.0676	-0.3471	0.0424	-0.3428	0.2048	0.0901	-0.5702	-0.3494	0.0543

*P<0.05;** P<0.01; *** P<0.001

thorax length, wing length and head width (all measured from slides). Abdomen length was excluded because it could not be measured in most specimens. All individuals were photographed at 0.5x magnification at the moment of marking. Morphological variables were obtained by measuring 3x projections of the slides with a digital caliper to the nearest 0.01 mm (Cordero, 1994). Thorax length was measured as the distance between the tubercule in the prothorax and the basal insertion of the abdomen; wing length was measured from the base to the tip of the hind wing and head width was taken as the maximum width of the head in dorsal view (fig. 1).

Individual values of absolute fitness were standardized to relative fitness, by dividing by mean fitness (Lande & Arnold, 1983). Morphological characters were normally distributed, and therefore were not transformed. They were standardized to mean=0 and variance=1 before analysis. By means of a multiple regression procedure with relative fitness as the dependent variable and phenotypic characters as independent variables, I estimated the selection gradients as the standardized partial regression coefficients (Lande & Arnold, 1983; Moore, 1990). Examination of residuals indicated normality in most cases. Therefore significance of selection gradients was calculated from the standard errors estimated with the multiple regression procedure. Taking into account that the probability level was in most significant cases <0.001, these deviations from normality were unlikely to change the significance.

Results

No significant directional selection gradient was detected on any phenotypic variable (table 1). Table 2 presents selection gradients that reflect nonlinear (stabilizing, disruptive) and correlational selection. In males date of emergence was under very intense non-linear selection at all selection episodes. Wing length was also under significant selection. In females only date of emergence and head width had highly significant selection gradients.

In males there was a significant negative correlational gradient between body and wing length for lifespan and visits/lifespan and a positive gradient between thorax length and date of emergence for matings/visit (table 2).

Discussion

The existence of variance in fitness places an upper limit on the force of selection that can act on any phenotypic character (Arnold & Wade, 1984a), but to demonstrate that selection is acting on one particular character, a relationship between fitness and the phenotypic character must be found. Several studies have shown a relationship between size and mating or reproductive success in odonates although other studies have

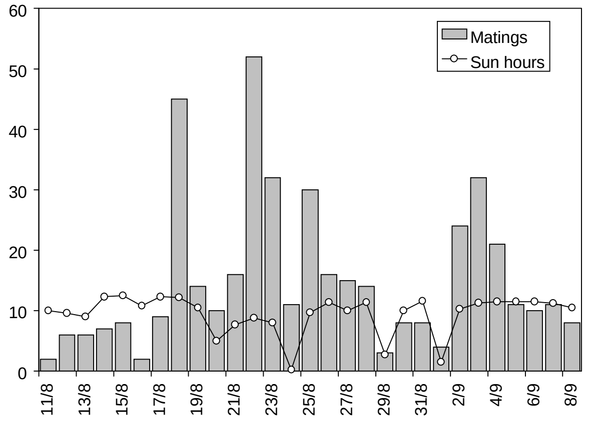

Figure 2. Daily variation in the number of matings observed and its relationship to weather.

indicated no relationship (Corbet, 1999). It is also common to find contrasting results if different morphological traits are analysed. For instance, Kasuya et al (1987) found direct selection for shorter hind wing length but larger abdomen in the dragonfly *Orthetrum japonicum*. A recently published meta-analysis suggests nevertheless that fitness is a positive function of body size in mature odonates (Sokolovska et al., 2000). This conclusion however might be premature as short-term and long-term studies were merged in this meta-analysis and phylogeny was not taken into account (D.J. Thompson pers. comm.). Furthermore, as the results of the present study indicate, the relationship between size and fitness is not easily described as a single correlation. In a previous paper (Cordero, 1995) I found that male body length was positively correlated with mating success in this population, but the multivariate analysis indicated that male size was not under directional selection (table 2). This contrasting finding might be explained by the positive correlation between date of marking and size ($r=0.25$, $p<0.001$), and suggests that multivariate analyses are needed to test for the effect of body size on fitness.

Furthermore, non-linear selection was also found on morphological characters (table 2) in many selection episodes, the most intense being on lifespan and visits/lifespan in males (table 2). In males significant non-linear selection in mating efficiency (sexual selection) acted on wing length. This could be related to the flying ability to search for females. Stabilizing sexual selection on morphological characters has also been detected in a dragonfly, *Libellula luctuosa* (Moore, 1990) and a fish, *Cyprinodon pecosensis* (Kodric-Brown & Hohmann, 1990). Stoks (2000) has recently shown stabilizing sexual selection in male body size in another scrambling damselfly, and Carchini et al. (2000) reported that small males of *Ischnura elegans* had a greater short-term mating success. Fitness does not therefore seem to be a linear function of body size in non-territorial odonates (but see Sokolovska et al., 2000).

Conrad and Pritchard (1992) have provided an ecological classification of odonate mating systems that attempts to develop predictions about the relative influence of intra- and inter-sexual selection on the evolution of different odonate mating systems. When the co-occurrence of males and females is predictable but when males are unable to control female access to oviposition sites, they predicted the existence of a “female-control” mating system. In this situation, males must actively seek mates and copulate, without a courtship display, for long periods. This mating system is appropriate for describing the reproductive behaviour of *I. graellsii*. Conrad and Pritchard (1992) predicted that under a “female-control” mating system, sexual selection on males should be on traits that affect their mate-seeking ability. The existence of non-linear selection on wing length in male mating efficiency could be an example of this kind of sexual selection.

The date of emergence (table 2) was under very intense disruptive selection in males at all selection episodes. This is a clear example of contrasting natural and sexual selection. It probably reflects the effect of climatic conditions on male mating probability (Thompson, 1997), and lifespan. Figure 2 shows that the number of matings observed in this population was maximum on 18 and 22 August, probably due to rainy conditions on some days. Males that did not visit the pond on these days had clearly a lower mating probability, but this effect was not so intense on females because they could mature eggs and lay them afterwards.

The interpretation of correlational gradients is difficult. A positive value indicates that traits are being selected to become positively correlated; the opposite is true for a negative value (Phillips & Arnold, 1989). Body and wing length were selected to become negatively correlated (table 2). The correlational selection gradient was significant only at two selection episodes, namely lifespan and visits/lifespan. Cordero (1994) found that wing length was isometric in male *I. graellsii* but showed positive allometry in females. The present results might explain this sexual difference in body proportions.

Estimates of heritability of body size in male and female *I. graellsii* are 0.4 and 0.5 respectively (Cordero, 1992b). Therefore, at least in some populations, there is additive genetic variation for adult body size. This is a prerequisite for a response to selection on this character. If additive variance for morphological characters was available during the period of study in this population, the observed selection pressure could then change the phenotypic distribution of characters in the next generation.

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References

Arnold, S.J. 1983. Sexual selection: the interface of theory and empirism. In: *Mate choice:* 67-107 (P.Bateson, Ed.).

Cambridge: Cambridge University Press.

Arnold, S.J. & Wade, M.J. 1984a. On the measurement of natural and sexual selection: theory. *Evolution,* 38:709-719.

Arnold, S.J. & Wade, M.J. 1984b. On the measurement of natural and sexual selection: applications. *Evolution,* 38:720-734.

Carchini, G., Chiarotti, F., Di Domenico, M. & Paganotti, M. 2000. Fluctuating asymmetry, size and mating success in males of *Ischnura elegans* (Vander Linden) (Odonata: Coenagrionidae). *Anim. Behav.,* 59:177-182.

Conrad, K.F. & Pritchard, G. 1992. An ecological classification of odonate mating systems: the relative influence of natural, inter- and intra-sexual selection on males. *Biol. J. Linn. Soc.,* 45:255-269.

Corbet, P.S. 1999. *Dragonflies. Behaviour and ecology of Odonata*. Essex, UK: Harley Books.

Cordero, A. 1987. Estructura de población en *Ischnura graellsii* Rambur, 1842 (Zygop. Coenagrionidae). *Boln. Asoc. esp. Entom.,* 11:269-286.

Cordero, A. 1989. Reproductive behaviour of *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica,* 18:237-244.

Cordero, A. 1990. The adaptive significance of the prolonged copulations of the damselfly, *Ischnura graellsii* (Odonata: Coenagrionidae). *Anim. Behav.,* 40:43-48.

Cordero, A. 1991. Fecundity in *Ischnura graellsii* (Rambur) in the laboratory (Zygoptera: Coenagrionidae). *Odonatologica,* 20:37-44.

Cordero, A. 1992a. Density-dependent mating success and colour polymorphism in females of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). *J. Anim. Ecol.,* 61:769-780.

Cordero, A. 1992b. Morphological variability, female polymorphism and heritability of body length in *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica,* 21:409-419.

Cordero, A. 1994. Reproductive allocation in different-sized adults *Ischnura graellsii* (Rambur) (Zygoptera: Coenagrionidae). *Odonatologica,* 23:271-276.

Cordero, A. 1995. Correlates of male mating success in two natural populations of the damselfly *Ischnura graellsii* (Odonata: Coenagrionidae). *Ecol. Entomol.,* 20:213-222.

Cordero, A. & Miller, P.L. 1992. Sperm transfer, displacement and precedence in *Ischnura graellsii* (Odonata: Coenagrionidae). *Behav. Ecol. Sociobiol.,* 30:261-267.

Cordero Rivera, A. & Egido Pérez, F.J. 1998. Mating frequency, population density and female polychromatism in the damselfly *Ischnura graellsii*: an analysis of four natural populations. *Etología,* 6:71-78.

Darwin, C. 1871. *The descent of man and selection in relation to sex*. Madrid: Edaf (Spanish edition 1982).

Fincke, O.M. 1986. Lifetime reproductive success and the opportunity for selection in a nonterritorial damselfly (Odonata: Coenagrionidae). *Evolution,* 40:791-803.

Grafen, A. 1988. On the uses of data of lifetime mating success. In: *Reproductive Success:* 454-471 (T.H.Clutton-Brock, Ed.). Chicago: University of Chicago Press.

Kasuya, E., Mashima, Y. & Hirokawa, J. 1987. Reproductive behaviour of the dragonfly *Orthetrum japonicum* (Odonata: Libellulidae). *J. Ethol.,* 5:105-113.

Kodric-Brown, A. & Hohmann, M.E. 1990. Sexual selection is stabilizing selection in pupfish (Cyprinodon pecosensis). *Biol. J. Linn. Soc.,* 40:113-123.

Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution,* 37:1210-1226.

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.

Phillips, P.C. & Arnold, S.J. 1989. Visualizing multivariate selection. *Evolution,* 43:1209-1222.

Stoks, R. 2000. Components of lifetime mating success and body size in males of a scrambling damselfly. *Anim. Behav.,* 59:339-348.

Sokolovska, N., Rowe, L. & Johansson, F. 2000. Fitness and body size in mature odonates. *Ecol. Entomol.,* 25:239-248.

Thompson, D.J. 1997. Lifetime reproductive success, weather and fitness in dragonflies. *Odonatologica,* 26:89-94.

Wade, M.J. & Arnold, S.J. 1980. The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. *Anim. Behav.,* 28:446-461.