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Field estimates of reproductive success in a model insect: behavioural surrogates are poor predictors of fitness, Thompson et al (2011) - SELF-ARCHIVED COPY

Title: Field estimates of reproductive success in a model insect: behavioural surrogates are poor predictors of fitness.

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ABSTRACT

Understanding, and therefore measuring, factors that determine fitness is a central problem in evolutionary biology. We studied a natural population of *Coenagrion puella* (Odonata: Zygoptera) over two entire breeding seasons, with more than a thousand individuals uniquely marked and genotyped, and all mating events at the rendezvous site recorded. Using a parentage analysis, fitness of individuals in the first generation was quantified as the numbers of offspring that survived to maturity. While mating behaviour can be predicted by environmental and demographic variables, the numbers of mature offspring produced (fitness) is largely unexplained and, crucially, is poorly correlated with behavioural observations of mating, particularly for females. Nevertheless, male fitness was positively related to mating behaviour while female fitness was positively related to lifespan and negatively related to ectoparasitic mite burden. Thus we demonstrate that behavioural measures of reproductive success are not necessarily reliable estimates of fitness in natural populations.

INTRODUCTION

Evolution acts upon the pool of available genetic variation, with the reproductive performances of different individuals determining their genetic representation in future generations: the central concept in natural and sexual selection. Estimates of reproductive performance are required to understand the variety and efficacy of selective processes that operate and the qualities that confer any reproductive advantage. Reproductive performance may be measured as "the total number of offspring produced by an individual during its lifetime that survive to join the mating population of the next generation". This is one of a number of definitions of fitness that appear in evolutionary biology literature and the one which we use here. Despite being an intuitive concept, quantifying fitness in natural populations remains challenging with key factors being the difficulties associated with following individuals throughout their entire lifespan and assigning all offspring produced by these individuals. With this in mind, perhaps the most productive studies of fitness have focused on large, terrestrial ungulates with low birth rates and relatively small population sizes that provide an opportunity to monitor breeding activity and maternal care over long periods, and even define pedigrees without recourse to genetic data (Kruuk et al., 1999; Hamel et al., 2009).

Of course, long-term field studies are rare (Clutton-Brock & Sheldon, 2010) and thus a spectrum of surrogates have been used to study fitness; these range from morphological traits that correlate with the probability of obtaining a mate, such as body condition (Jakob et al., 1996) or body size (Simmons, 1988; Sokolovska et al., 2000), to behavioural measures of copulatory success that are assumed to correlate with offspring number, such as daily mating rate (DMR) (average number of matings per day throughout reproductive life; *e.g.* Thompson & Banks, 1989) and lifetime mating success (LMS) (total number of matings obtained throughout lifetime; *e.g.* Fincke, 1982; Stoks, 2000). Typically, each of these surrogates is considered to come progressively closer to fitness. The pre-genetic analysis state of the art was synthesised by Clutton-Brock (1988) and even in this volume Grafen (1988) highlighted gaps between these surrogates and the actual numbers of offspring surviving to reproduce. Subsequent molecular-genetic studies have uncovered frequent cryptic mating behaviour in natural populations (Kraaijveld-Smit, 2008) that question the accuracy of behavioural observations of reproductive success in many instances. Only through a thorough genetic analysis can parentage be determined unequivocally, an essential requirement in bridging the gap between fitness surrogates and fitness itself. Such an approach has been demonstrated for a range of vertebrates, with studies on ungulates in particular illustrating the importance of factors such as body size, population density, lifespan, time during the season and environmental conditions upon successful production of offspring (Albon et al., 1987; Coltman et al., 1999; Kruuk et al. 1999; Gaillard et al., 2000).

By contrast, estimates of fitness in wild insect populations are lacking, despite numerous and detailed studies of reproductive behaviours that infer fitness based on DMR and LMS. One pioneering study in semi-natural conditions was that of Fincke & Hadrys (2001) who used genetic markers to allocate 42 offspring to 48 wild-caught mating pairs of the tropical damselfly *Megaloprepus coerulatus*. Although broods were raised in large, artificial containers, which likely minimised natural mortality rates, fewer than half of the mated pairs produced any offspring and there was no relationship between common predictors of fitness (*e.g.* body size, clutch size). Instead, female reproductive success was related to the time span over which eggs hatched, possibly as a strategy to limit cannibalism. Only recently has one study, on crickets (*Gryllus campestris*), used a combination of video-monitoring and molecular estimates of parentage to uncover certain life history components of fitness under natural conditions (Rodríguez-Muñoz et al., 2010). Rodríguez-Muñoz and co-workers allocated 184 offspring to 149 parents and showed that larger, longer-lived individuals had more mates and higher numbers of offspring. An emerging theme from

both studies is that increased mating success does not necessarily result in greater numbers of offspring. It is important to clarify the relationship between mating success and offspring number, given the previous reliance of LMS (in particular) studies on the assumption that the two are strongly related. In addition, a number of additional factors (listed below) have been hypothesised to explain the variation in fitness in wild insect populations. These have rarely been tested in a natural population using a combination of behavioural monitoring and molecular parental allocation.

Odonates (dragonflies and damselflies) are used extensively as model organisms in a range of ecological and evolutionary contexts, particularly with respect to LMS (see Koenig, 2008 for review) as they can be captured, handled, marked and recaptured and/or observed easily from distance (Stoks, 2000; Cordero-Rivera & Stoks, 2008). We monitored the reproductive behaviour of all adult damselflies (*Coenagrion puella*: Zygoptera: Odonata) at a breeding site for two generations and, using genetic data, allocate offspring to 425 parents. These data are used to determine the influence of factors that are explicitly stated to impact upon insect, particularly odonate, fitness, including (1) environmental conditions (Thompson, 1990), (2) body size (Sokolovska et al., 2000) and (3) ectoparasite burden (Andrés & Cordero, 1998; Forbes & Robb, 2008). We also test the effects of additional variables that have been implicated in mediating fitness in other taxa: (4) population density, (5) mature adult lifespan, (6) time of season and (7) energy invested in reproductive activity. Despite extensive studies of odonate reproductive behaviour this is the first study of fitness in a natural population in this taxon, and to our knowledge, only the second such study in insects. Indeed, studies involving our model species, *C. puella*, have been cited extensively as examples of LMS studies (Banks & Thompson, 1985, 1987), which makes the evaluation of the link between fitness surrogates and fitness particularly illuminating.

The aims of this study are (1) to quantify the correlation between surrogate measures of fitness (DMR, LMS) and fitness, defined as the number of offspring surviving to maturity (FIT), and (2) describe the relative influence of putative key factors in determining fitness in males and females, and compare them to theory and previous studies.

METHODS

Study organism and study site

Coenagrion puella L. (1758) is among the best understood odonates in terms of its reproductive biology (Banks & Thompson, 1985, 1987; Thompson, 1990; Lowe et al., 2009). *C. puella* is univoltine throughout most of its range, including southern England. Males are not territorial but gather at mating rendezvous (usually well-vegetated, lentic habitats) and compete in scramble competition for females. Two intensive capture-mark-recapture studies were performed on a population of *C. puella* at a pond in southern England (50°57'39"N, 0°58'41"W) during 2005 and 2006. The pond (~32x14m and 1.5m deep) is isolated from other water bodies by ~1km, rendering the influence of immigration negligible. During the entire flight season (11th May–30th July 2005 and 17th May–29th July 2006) between three and six people were present at the study site every day throughout the hours of reproductive activity (~09:30–16.30 hours). All unmarked individuals were caught with a kite net and marked with a small dot of paint on the dorsum on the thorax and given a unique alphanumeric code on the left hindwing using a permanent marker. All reproductive activity by *C. puella* at the site was recorded: identifications were made directly by eye where individuals were accessible or using close-focussing binoculars.

Phenotypic, demographic and behavioural measurements

At first capture, we measured body size (forewing length to the nearest 0.1 mm using digital callipers), ectoparasite burden (the number mites, all *Arrenurus* spp., described by Hassall et al., 2010) and removed

the middle-left leg for genetic analysis. Male and female population sizes were the number of different individuals present on each day; the mean value of these daily population sizes was calculated over the lifespan of each individual. We also calculated (1) mature adult lifespan (number of days between the first and last sighting), (2) two proxies for effort invested into reproductive activity - the number of days on which each individual was recorded at the pond and time of arrival at the pond and (3) time in the season (the first and last days that individuals were recorded at the pond). Daily mating rate (DMR) was defined according to sex. For males, DMR was the total number of females with which each male was observed exhibiting reproductive behaviour divided by that individual's lifespan; for females, DMR was the proportion of days on which the female achieved matings. The reason for this distinction is that, while male mating success is typically assumed to be related to offspring production achieved through multiple mating, females able to fertilise an entire clutch of eggs with the sperm from a single mating will not necessarily benefit further. Lifetime mating success (LMS) for males was the total number of matings achieved and for females was the total number of days on which matings were achieved. All variables were examined to assess normality using Shapiro-Wilk tests in R (R Development Core Team, 2010) and either square-root transformation (positive skew) or square-transformation (negative skew) were employed where necessary.

Environmental data

Data for the following climate variables were obtained from the UK Meteorological Office weather station (51°7'26"N, -1°26'24"W, 37km from the study site) for each day during the two field seasons: total daily sunshine (hours), mean daily temperature (°C) and total daily precipitation (mm). The mean value for each climate variable was calculated over the lifespan of each individual in the study and transformation was applied where necessary (see above).

Genotyping and parentage analysis

DNA was extracted from leg samples using a standard high-salt protocol and every individual was genotyped at 12 variable microsatellite loci (Watts et al. 2004; Lowe et al., 2007) using an ABI3130. As parentage assignment is sensitive to genotyping error, we genotyped every sample at least twice; full details of the genotyping procedures are described elsewhere (Lowe et al., 2009). Parentage of the mature individuals in 2006 was determined from the pool of 2005 individuals using CERVUS v.3.0 (Kalinowski et al., 2007). Briefly, we ran simulations to estimate critical LOD scores and values of delta that are used to delineate acceptable parent-offspring triads (at 80% confidence, see Marshall et al., 1998; Kalinowski et al., 2007 for details); we assumed that 95% of the parental generation was sampled (*n*=500), a genotyping error rate of 0.5% and 650 offspring (roughly the numbers of individuals caught in 2005 and 2006 respectively). Parentage assignments were conducted using the same parameters and allocations made from the most likely (based on delta scores) parent-pairs; these allocations were checked against field observations and rejected if the life spans of the proposed parents did not overlap (with a miss-match of ±3 days to allow for unobserved activity) and the next best parent-pair was selected. These parent-offspring allocations provide an estimate of fitness (FIT) for every 2005 individual.

Predicting fitness measures

Stepwise regression was used to construct general linear models which explained the greatest proportion of the variance in the three response variables: DMR, LMS and fitness (=FIT). Twelve core predictor variables were available for selection: mean hours of sun, mean daily temperature (°C), mean daily precipitation (mm), mean male population size, mean female population size, mean arrival time, lifespan (days), days spent at the pond, mite count, wing length (mm), first day at the pond and last day at the pond. Additional models were created with (1) DMR added to the pool of variables to explain LMS and FIT,

and (2) both DMR and LMS added to the pool of variables to explain FIT (see Table 1 for model details). Quadratic terms were not included because they showed high co-linearity with the linear terms and this would have substantially increased the complexity of models.

Selection of terms in the models was based on minimising Akaike's information criterion (AIC) using the "stepAIC" function in the MASS package (Venables & Ripley, 2002) in *R* (*R* Development Core Team, 2010). Model selection was performed twice: once beginning with an empty model and once beginning with the full model. Where these two iterations produced different results, the final model was selected by choosing the model with the lowest AIC. Models were first run with DMR, LMS and FIT as response variables and only the 12 core variables mentioned above as predictors. Secondly, LMS and FIT were analysed with core predictors plus DMR added to the variable pool. Finally, FIT was analysed with the 12 core variables, DMR and LMS in the variable pool. This gave a total of 18 models (Table 1). Pearson correlations were used to assess agreement between the three fitness measures.

Intersexual variation in reproductive skew
Reproductive or mating success distributions are frequently skewed to such an extent that transformation is insufficient to conform to the assumptions of parametric statistics. Such is the case here and so we use the randomisation methods of Rodríguez-Muñoz et al. (2010) to compare male and female mean and variance for our three fitness measures; resulting *P*-values are the proportion of 10,000 randomisations greater than or equal to the observed value.

RESULTS
Over two entire flight seasons we marked, observed and genotyped 1,036 damselflies (*n*=425 and 611 in 2005 and 2006 respectively). During some 1,085 hours of observation, 15,199 behavioural records were taken, including 9,444 instances of mating behaviour (tandem position, copulation or oviposition).

Parentage
The mean genotyping error rate across all loci was 0.18% (range 0.0-0.24%). Most (>99%) instances of genotyping error were a result of allele dropouts in one of the two genotyping rounds and the few ambiguous genotypes were resolved by further PCRs. Initially, we identified statistically credible (based on delta scores) parent-pairs for 97% of the offspring; however, 156 of these parent pairs had non-overlapping lifespans, so we reallocated "their" offspring to the second best parent pair when they had overlapping lifespans. This procedure left 62 individuals from 2006 who could not be assigned parent-pairs that were consistent with both genetic data and field observations on adult lifespan. Thus, using a combination of genetic data and field data we assigned parents to 549 (90%) individuals from the 2006 generation.

Modelling fitness components
GLMs describing variations in DMR explained between 42% and 62% of the variation in the data (Table 1, Figure 1). Increasing ectoparasitic mite burdens lowered DMR in both sexes, although this was not the case for females in 2006. DMR was positively related to the number of days spent at the mating rendezvous. Higher male population sizes negatively affected male DMR while positively affecting female DMR. For both sexes in both years, DMR was negatively related to lifespan; this may be the result of a greater proportion of climatically unsuitable days in longer-lived individuals. Males arriving later achieved a higher daily mating rate, likely as a result of females also arriving later (data not shown).

A greater proportion of the variation in LMS was explained than was the case for DMR and the proportion of variation explained was greater in females (90-93%) than males (67% in both years; Table 1, Figure 1).

For DMR, male population size positively affected female LMS while negatively affecting male LMS, the number of days spent at the mating rendezvous was positively related to LMS in both sexes and mites negatively affected mating rates in both sexes. When DMR was added as a variable in the models, this was highly positively related to LMS. However, accompanying the addition of DMR was an affect of phenology on LMS. Females living in the peak of the flight season (later first date and earlier last date) experienced higher LMS while the opposite (earlier first date and later last date) increased LMS in males.

Explanatory power of models describing FIT was far lower than for DMR or LMS (28-34%, Table 1, Figure 1). In males, FIT was consistently positively related to female population size and mating success (when included in the models). DMR was negatively related to FIT when LMS was included in the models. The effect of phenology seen in LMS also appeared in FIT models involving environmental variables and was strengthened by the addition of DMR. Female FIT was never related to mating success. Instead, FIT in females was positively related to lifespan and negatively related to ectoparasitic mite burden regardless of whether mating data were included as predictors.

Correlations between fitness measures
Correlations between behavioural fitness measures (DMR and LMS) were positive and high for males in both years and females in 2005 (Table 2). Females in 2006 exhibited a strongly unimodal relationship between DMR and LMS and thus the relationship was better described using a quadratic regression (DMR, $F=13.8$, $P<0.001$; DMR^2, $F=217.3$, $P<0.001$; $R^2=0.470$), with peak LMS occurring at a DMR of around 0.6; the correlation coefficient of this relationship brings the 2006 female data into line with the data for females in 2005 and males in both years. LMS was significantly correlated with FIT in both sexes (males, $r=0.513$, $P<0.001$; females, $r=0.468$, $P<0.001$) while the relationship between DMR and FIT was significant but weaker in both sexes (males, $r=0.297$, $P<0.001$; females, $r=0.182$, $P=0.022$).

Mating and reproductive skew
In 2005, the difference between DMR and LMS of males and females was statistically non-significant, while in 2006 males achieved a higher DMR and females achieved a slightly higher LMS (Table 3). These figures, while defined differently for each sex, are comparable because they equate to the number of fertilised clutches of eggs per individual. As expected from sexual selection theory, the variance in male mating success was generally higher (DMR in both years, LMS in 2006). However, females achieved a far greater average number of offspring (FIT) than males as well as exhibiting a significantly higher degree of variance in the number of offspring.

DISCUSSION
Quantifying fitness in wild populations is complex, largely due to inherent difficulties in making detailed field observations and accurately allocating progeny to each individual, particularly when life history includes stages that are inaccessible (*e.g.* aquatic larvae) and/or highly mobile (*e.g.* flying adult), and there is an absence of parental care. Nonetheless, using an insect model that is tractable to *in situ* observations of mating behaviour, we genotyped a large (*n*>400) population to determine individual variation in fitness and quantify the relationship between surrogate measures of fitness based on observed reproductive behaviour and fitness: while mating behaviour is predicted well by environmental and demographic variables, a substantial proportion of the variance in fitness, *i.e.* numbers of offspring surviving to maturity, is left unexplained. Nonetheless, the portion of fitness that can be explained differs between sexes and is in line with theory: male fitness is determined by mating success while female fitness is determined by lifespan and ectoparasitic burden.

Studies attempting to infer advantages of certain phenotypes in the field often use behavioural observations of mating success as a surrogate measure of fitness (Stoks, 2000; Lappin & Husak, 2005; Stein & Uy, 2006). By integrating behavioural and genetic data we demonstrate two important points: first, the choice of behavioural measure is important. Correlations between DMR and FIT are significant but weak for both sexes suggesting that short-term mating success does not reflect fitness; indeed, there is a complex relationship between DMR and LMS in females in 2006 and a highly significant, negative effect of increasing DMR on male FIT in 2005 (Table 2). Second, while LMS significantly correlates with FIT in both sexes, a substantial proportion of the variance in offspring number (74% in males and 78% in females) remains unexplained. Neither DMR nor LMS were included in models of female FIT (Table 1), suggesting that other factors better explain the variation in offspring number. An obvious problem with a purely behavioural approach is the widespread evidence for cryptic mating behaviour (Kraaijveld-Smit, 2008). This is evident here as only 30% of assigned parent pairs were observed engaged in mating behaviour during the study (P.C. Watts, C.D. Lowe & D.J. Thompson, unpublished); as such these data challenge the traditional view of mating behaviour by damselflies where mating takes place predominantly at rendezvous (*i.e.* oviposition) sites (Corbet, 1999). Behavioural measures of mating must be treated with caution when used as indicators of fitness, particularly for female damselflies (see *e.g.* Cordero et al., 1998).

Male fitness can be partially explained by copulatory behaviour (LMS and DMR) and the number of available females (Table 1). Irrespective of whether the observed pairs *per se* successfully produce mature offspring, the former may reflect the success of generally more active males and is concordant with Bateman's expectation that, when low paternal investment is required to produce offspring, male fitness increases with the number of mates acquired (Bateman, 1948). Since male *C. puella* undertake scramble competition for females, it is not surprising that male FIT increases with female population size. Interestingly, while it is widely assumed that male LMS is strongly associated with male FIT, this may be limited by energetic costs (*e.g.* through sperm exhaustion, Warner et al., 1995). Potential evidence of this may be inferred from the positive correlation between DMR and LMS (Table 2), but they have opposing effects when considered together in a model explaining FIT (Table 1); the implication is that more matings increase male fitness, but these must be staggered over the male's lifespan. In addition, ectoparasite burden affects mating ability in male damselflies (Andrés & Cordero, 1998; Forbes & Robb, 2008), but this does not significantly impact upon fitness (Table 1). A similar observation may be made for intrasexual competition, whereby LMS is lower when male population density is higher (Stoks, 2000) but ultimately this does not significantly impact upon male fitness (Table 1). Finally, reproductive success has been suggested to correlate with body size in male odonates (Sokolovska et al., 2000). While an alternative mechanism of stabilising selection on body size has not been explored in most studies, it has been shown to be present in those studies with large sample sizes (Stoks, 2000; Thompson & Fincke, 2002). We see no evidence of an effect of body size on fitness anywhere in the analysis (see also Fincke & Hadrys, 2001 for females).

Mating success does not feature in models of female FIT (Table 1). By implication mating success is a proxy for the number of different mates since females were rarely observed re-mating with the same male. Thus our data are more consistent with studies that find little obvious benefit to polyandry (Fincke, 1994) than any of the hypotheses relating to putative benefits of multiple mating (Arnqvist & Nilsson, 2000). More importantly, since female *C. puella* only visit breeding sites when they are ready to lay eggs, LMS reflects the total number of zygotes produced (Banks & Thompson, 1987); hence, this result is somewhat counterintuitive as it implies that the numbers of offspring surviving is decoupled from the number of fertilised clutches. Substantial variance in clutch survivorship is a common outcome when odonate larvae are raised in artificial conditions (Fincke & Hadrys, 2001; Bots et al., 2010), likely reflecting the importance of intraspecific competition and/or predation. Beneath an overriding pattern of "unpredictable offspring

survivorship", the best predictor of fitness of female *Megaloprepus coerulatus* was the extent to which egg hatching was staggered (up to 84 days between the first and last eggs present) within a clutch, likely increasing the chance that some offspring hatch in window of reduced competition/predation (Fincke & Hadrys, 2001). *C. puella* egg hatching occurs within a limited period (12 days post-oviposition) with little variation (Waringer & Humpesch, 1984) so this is unlikely to affect our results. Intriguingly, female lifespan correlates with FIT in *C. puella*. We hypothesise that female lifespan reflects maternal condition and this has a greater impact on fitness rather than total fecundity *per se*; indeed, there is evidence that breeding *C. puella* females senesce at a slower rate than non-breeding females (C. Hassall, T.N. Sherratt, P.C. Watts & D.J. Thompson, unpublished). Further, lower temperature, increasing age and parasitism all negatively affect clutch size in odonates (Banks & Thompson, 1987; Forbes & Baker, 1991; Córdoba-Aguilar et al., 2003), with parasites reducing longevity in some, but not all (Andrés & Cordero, 1998; Forbes & Robb, 2008), species. Although temperature had little effect in our models, mite burden did impact on female fitness. Ectoparasites may have a direct (clutch size reduction) and/or indirect (reduced numbers of clutches) effect on total lifetime egg production, based on the energetic cost of either enduring (loss of nutrients to the engorging mite) or fighting (melanic encapsulation of mite feeding tubes) ectoparasitic assault, but, as noted above, there is only a moderate correlation between clutch size and fitness in damselflies. Overall, the combination of fewer ectoparasitic mites and a longer female lifespan should lead to a greater overall fecundity, with clutches being laid over a longer time period perhaps providing the crucial increase in fitness.

One outcome of this study is that apparently greater reproductive success, mediated by variation in population density, lifespan and the number of days spent at the mating rendezvous, does not necessarily translate into enhanced survival of offspring that attain maturity. Thus, it appears that fitness is poorly predicted by adult traits and the weather during the reproductive season. Assuming some heritable genetic variation, the efficacy of selection to act on adult traits (such as fecundity, longevity) depends upon the extent of genetic correlations between determinants of parental success (survival and acquiring mates in a terrestrial habitat) and offspring survival during the larval stage. With an average of 200 eggs per clutch (estimated for *C. puella* from Banks & Thompson, 1987), then the 425 observed ovipositions in 2005 would produce some 85,000 eggs; in 2006, 611 reproductively-active adults were marked, meaning that there is ~0.7% egg-to-adult survival rate in this population with most mortality occurring during the aquatic larval phase. Indeed, the larvae are subject to a range of selection pressures that relate to a host of intraspecific and/or interspecific interactions. For example, selection may act on larvae to encourage greater size and territoriality which may result in benefits over conspecifics upon emergence (Harvey & Corbet, 1985); of course, where there is a substantial size difference between larvae or larval densities are high cannibalism can substantially reduce larval survivorship (Buskirk, 1989; Anholt, 1994; Corbet, 1999). High rates of early mortality may result in selection for late season eggs to develop quickly and emerge earlier in the next season (Lowe et al., 2009). Hence, while adult body size neither correlates with mating success nor realised fitness, larval body size is likely correlated with survival. The potential correlations, or antagonism, between larval and adult traits that determine success (survival and subsequent mating) warrants further study. Indeed, upon reaching maturity, 201 out of 263 (76%) males and 134 out of 159 (84%) females produced at least one offspring: it could be argued from this that the opportunity for selection in adults is relatively small, since the probability of producing an offspring is reasonably high.

Sexual selection theory states that the sex with the lower investment in reproduction should have the greater skew in reproductive success (Bateman, 1948). While this is true in terms of mating success in our system, with males exhibiting higher degrees of skew where significant intersexual differences occur, it is females who have the greater skew in fitness (FIT, Table 3). This runs counter to previous findings

(Rodríguez-Muñoz et al., 2010) and has implications for predictions about the efficacy of sexual selection in odonates with a similar life history. There could be a number of explanations for this. The first is that the spatial clustering of eggs renders large portions of each clutch susceptible to mortality, for example, through parasitism, predation, cannibalism or simply damage to the vegetation; indeed, an extreme form of this spatially-correlated offspring mortality could occur if the water in which the female oviposits does not reach the temperature required for embryonic development (12°C in *C. puella*, Waringer & Humpesch, 1984). A second explanation is that suitable weather conditions for reproduction are temporally correlated. Thus, an otherwise-high quality female may have no opportunity to reproduce if weather is unsuitable (Thompson, 1990): indeed, Thompson (1990) went so far as to state that "lifetime egg production is largely determined by chance" in *C. puella* females.

To conclude, we use a combination of behavioural and genetic data to provide the first calculation of fitness using parentage assignment for an entire odonate population. The results demonstrate that behavioural surrogates of fitness are not necessarily the best predictors of actual reproductive output, particularly for females. Furthermore, this population (and probably many other odonates) fail to follow Bateman's principle of higher reproductive skew in males. Finally, we provide the first evidence of an effect of parasitic mites on female fitness in odonates, as well as confirming the positive effects of mating success and lifespan on the fitness of males and females, respectively. Future studies wishing to measuring fitness should at least take care to validate behavioural surrogate measures before relying too heavily upon them and should ideally include molecular assignment of offspring to parents to quantify fitness.

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Table 1. Coefficients from general linear models (rows) explaining variation in fitness measures (DMR=daily mating rate, LMS=lifetime mating success, FIT=fitness) of male and female *Coenagrion puella* for two seasons using a combination of 12 core predictor variables and behavioural surrogates of fitness (DMR and LMS) (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

| | | | Core Predictor Variables | | | | | | | | | | | | Fitness surrogate | | |
| | | | Environment | | | | Population size | | | | Time in season | | | | | | |
Response	Sex	Year	Sun (hrs)	Temp (°C)	Prec (mm)	Size (mm)	Mites	Male	Female	Lifespan (days)	First day at pond	Last day at pond	Arrival time	Days at pond	DMR	LMS	R^2
DMR	M	2005		0.036***			-0.027***	-0.004**		-0.247***	0.006*		0.001*	0.492***			0.418
	M	2006					-0.017**	-0.019***	0.019***	-0.573**		-0.017*	0.001**	1.314***			0.417
	F	2005					-0.027*	0.009***		-0.173***		0.010***					0.510
	F	2006		-0.017**					0.006***	-0.394***	-0.021***	0.0002***		0.314***			0.623
LMS	M	2005		0.074***			-0.033**	-0.010***		-0.420***		0.013**	0.002*	1.479***			0.669
	M	2006	-0.019*				-0.017*	-0.010***	0.015***				0.001**	1.650***			0.667
	F	2005					-0.040*	0.012***			0.086*	-0.074*		0.626***			0.897
	F	2006						0.005**			-0.003*			0.976***			0.934
	M	2005			0.094**					-0.429***	-0.086***	0.092***		0.697***	1.602***		0.920
	M	2006			0.063***	-0.018*				0.305***	-0.021***	0.0197***		0.233**	1.108***		0.962
	F	2005								0.766***	0.101***	-0.102***		0.507***	1.347***		0.967
	F	2006	-0.009*						-0.003***	0.492***	0.023***	-0.0003***		0.599***	1.248***		0.977
FIT	M	2005							0.010*			0.049*		0.296*			0.277
	F	2005	-0.047*				-0.065***	0.016***		0.311***							0.330
	M	2005							0.013**		-0.082***	0.085***			0.310**		0.286
	F	2005	-0.047*				-0.065***	0.016***		0.311***							0.330
	M	2005							0.011**						-0.852***	0.669***	0.339
	F	2005					-0.071***	0.031***		0.318***	-0.016**						0.333

Table 2. Pearson correlations between different measures of fitness in male (below diagonal) and female (above diagonal) *Coenagrion puella*. DMR=daily mating rate, LMS=lifetime mating success, FIT=fitness. Numbers in brackets are for the 2006 season.

	DMR	LMS	FIT
DMR		0.508 (0.686*)	0.182
LMS	0.842 (-0.776)		0.468
FIT	0.297	0.513	

*For females in 2006, the value is for a quadratic regression between DMR and LMS (see *Results* for details).

Table 3. Differences between sexes in the mean and variance of fitness components in *Coenagrion puella*. (DMR=daily mating rate; LMS=lifetime mating success; FIT=fitness; *P*, probability that mean or variance in fitness component differs between sexes).

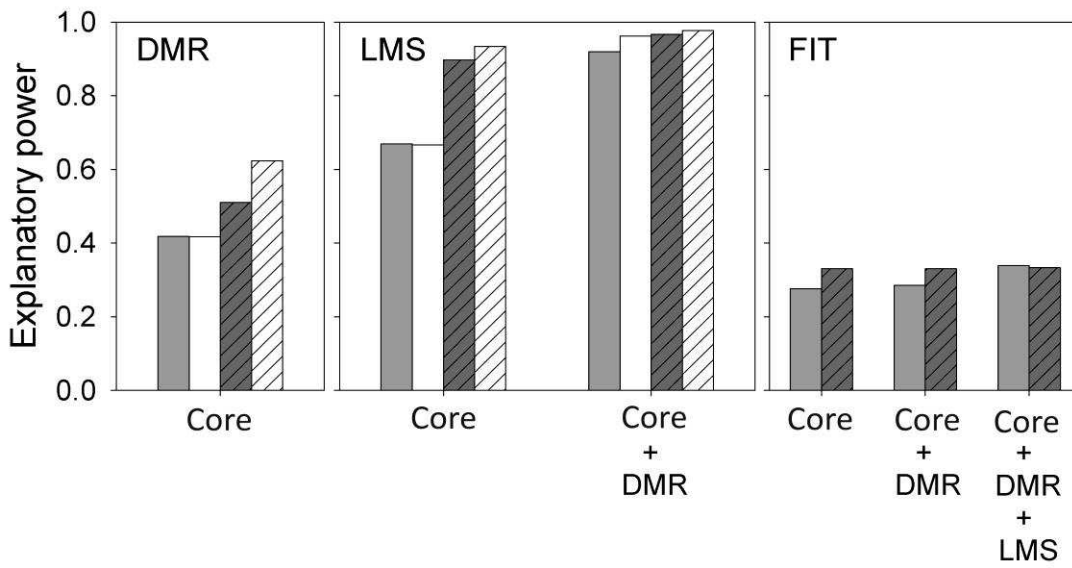| Fitness measure	Year	Male mean (SE)	Female mean (SE)	P	Male variance	Female variance	P
DMR	2005	0.480 (0.06)	0.511 (0.03)	0.645	1.096	0.121	<0.001
	2006	0.761 (0.06)	0.589 (0.02)	0.003	1.130	0.064	<0.001
LMS	2005	2.490 (0.21)	3.509 (0.28)	0.002	11.717	12.543	0.614
LMS	2006	5.057 (0.30)	5.799 (0.27)	0.038	30.393	18.355	0.002
FIT	2005	2.167 (0.15)	3.522 (0.26)	<0.001	5.850	11.023	0.002

Figure 1. Proportion of variation explained in the fitness of *Coenagrion puella* using models containing different sets of predictors (Core=12 core variables – see Table 1; DMR=daily mating rate; LMS=lifetime mating success; FIT=fitness, measured by numbers of mature offspring produced). Dark bars are 2005 and white are 2006, open bars are males and shaded bars are females.