Reproductive timing and patterns of development for the damselfly Coenagrion puella in the field

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Abstract. By a combination of detailed behavioral observations and molecular genetic approaches we have assessed development time, timing of first maturity, and the extent of genetic structure through the flying season in a wild population of the damselfly Coenagrion puella in England. This work provides the first estimate of development time (egg to mature adult) in the field based on individual damselflies. Development time was significantly longer for females than males. In contrast to reported laboratory studies, there was no difference in development times between different female color morphs. Development time ranged between 347 and 396 days and was negatively correlated with egg-laying date. As a result eggs laid early in one season reach adult maturity relatively late in the next; concurrently individuals developing from eggs laid late mature relatively early. We speculate that this pattern of development is a direct physiological response to seasonal environmental variation and results in reproductive synchrony within a population. Size, specifically hind wing length, declined with development time in males, but not in females. In one of the two years of the study there was evidence for weak clustering of related individuals during the reproductive season. This appeared to be the result of developmental synchronization within families: variance in timing of maturation was smaller in full-sib families than in half-sib families or randomly assigned unrelated groups.

Key words: Coenagrion puella; complex life cycle; damselfly; development time; fitness; life history plasticity; parentage analysis; temporal genetic structure; time constraints.

INTRODUCTION

Ever since Moran’s (1994) review, increasing attention has been focused on animals with complex life cycles. Animals that come into this category were defined by Moran as those whose lives contain different phases that “exhibit contrasting morphological, physiological, behavioral, or ecological attributes.” The literature on complex life cycles has focused in two areas: on the ecological, in which there has been an emphasis on how different stages evolve to gather resources while other stages disperse and reproduce; and the developmental, which has been concerned with how evolution in one phase effects development in another (Moran 1994). Although most subsequent ecological work has been carried out with holometabolous insects, such complex life cycles are not restricted to insects but are found in many groups such as amphibians, crustaceans, and mollusks.

The life history consequences of such life cycles for odonates were first explored by Johansson and Rowe (1999), building on theoretical work on life history constraints by Roff (1980, 1992) and Rowe and Ludwig (1991). Johansson and Rowe (1999) demonstrated that time-constrained larvae accelerated development rate and matured at an earlier age and at a smaller size. The body of work stimulated by Johansson and Rowe’s paper has indicated that under a time constraint organisms increased their development rate (e.g., Johansson et al. 2001, De Block and Stoks 2003), but results on body size were equivocal, contrary to the predictions of optimality models (e.g., Abrams et al. 1996). Detailed experimental work by De Block and Stoks (2005) indicated that larval constraints did not necessarily carry over into adult fitness through size and timing of transition. Roff et al. (2004) demonstrated that time constraints decoupled the relationship between age and size at maturity in a damselfly, particularly in relation to some physiological traits, most notably a component of immunity. They suggested that the predictive value of traits such as age and size at maturity might be restricted. Strobbe and Stoks (2004) pointed out that genetic constraints might have contributed toward different responses to a time constraint for size and mass.

While the mechanisms by which developmental constraints act and the responses of developmental parameters to time cues have remained unclear, it is apparent that development has important consequences for individual survival and fitness by determining the timing of life history transitions (e.g., maturity and reproduction; Plaistow and Siva-Jothy 1999). Maturity
must occur within a period during which the environment is suitable for reproductive activity, else the fitness of an individual is zero; however, within this window the relative timing of reproduction (e.g., early vs. late) may also have fitness consequences (see Discussion and Thompson 1997). Ultimately, understanding fitness effects associated with life cycle timing, and the evolutionary significance of variation in reproductive timing and temporal phenotypic clines requires direct estimates of (1) the persistence of temporal breeding structures, i.e., do “early” breeding parents produce early breeding offspring, and (2) the mechanisms by which relative reproductive timing may be maintained e.g., via developmental timing responses or trait heritability.

Variation in breeding times and associated temporal phenotypic clines may result from a range of mechanisms, which potentially reflect different evolutionary pressures. Relative reproductive timing may be strongly heritable as a result of divergent selection for differing reproductive strategies (e.g., “early” vs. “late” reproductive timing); temporal phenotypic clines might then arise as a result of adaptation by individuals reproducing at different times during the breeding season. Such a scenario can result in limited gene flow (via persistent nonrandom mating) through the reproductive season and distinct patterns of temporal genetic differentiation (Hendry and Day 2005). For example, in populations of the Pacific salmon *Oncorhynchus nerka* reproductive timing is strongly heritable (Hendry et al. 2004) as a result of selection for specific early and late reproductive strategies; the subsequent persistent mating structure restricts gene flow along the breeding season and temporal genetic structure occurs (termed “isolation by time” to reflect the analogy with isolation by distance [IBD] models of spatial genetic structure; see Methods and Hendry and Day 2005). Alternatively reproductive times may be plastic and determined by developmental timing and/or developmental constraints; temporal phenotypic clines might then arise if reproductive time is influenced by phenotypic traits (e.g., body size or energy stores) or if trait expression is influenced by the conditions experienced at the chosen reproductive time (Borash et al. 1998).

Currently, estimates of parameters such as development time and heritability of phenotypic traits from wild odonate populations are absent as these rely on tracking individuals over long time periods and establishing links between parents and offspring, which has traditionally been intractable for organisms with cryptic life history stages. However, the increasingly routine application of polymorphic genetic markers and critically the development of statistical approaches for the allocation of parent–offspring relationships in the wild have provided a powerful tool to estimate important life history parameters and examine evolutionary processes in natural populations (Kruuk 2004, Garant and Kruuk 2005).

Here, we use a combination of field observations and genetic approaches to estimate timing of reproductive maturity and temporal genetic structure in consecutive generations of a population of the damselfly *Coenagrion puella* (Coenagrionidae). Specifically, we test for plasticity between development time and date of maturity and the potential trade-off between development time and size in relation to date of maturity; we do this with respect to sex and female morph by tracking individuals genetically in the field. In addition, we make the first field-based estimates of individual development time and heritability of timing of maturity and adult body size for a natural odonate population. These parameter estimates provide a useful insight into the evolutionary processes shaping reproductive timing in an abundant damselfly.

**Methods**

**Study site and data collection**

We studied *Coenagrion puella* from a pond at Queen Elizabeth Country Park (QECP; see Plate 1), southern England (57°50′36″ N, 0°58′48″ W, elevation 104 m) that is isolated from other water bodies by ~1 km effectively excluding immigration to the site (Banks and Thompson 1985, Conrad et al. 1999). The pond has a maximum length, width, and depth of 32, 14, and 1.5 m, respectively, and the perimeter is ~75 m. There has been a pond at this site for many years, but the present pond was restored completely in March 2000. Additional details of the study site are given in Gillingham et al. (2007).

From 11 May to 30 July (in 2005) and from 17 May to 29 July (in 2006) between three and six people were present at the study site every day from 09:30 hours until the last individual of *C. puella* was active (typically between 15:30 and 17:00). Every individual *C. puella* was caught using a kite net, marked with a small dot of paint on the dorsum on the thorax (making it easier to identify previously caught individuals from distance), and given a unique number on its left hind wing. For newly captured individuals we recorded the sex, color morph (female phenotypes are either green, termed gynomorph, or blue resembling the color of the males, termed andromorph), and the length of the hind wing (using digital calipers). To provide a source of DNA for genotyping, the middle left leg of each individual was removed; removal of single damselfly legs does not measurably affect fitness (Fincke and Hadrys 2001; D. J. Thompson, unpublished data).

All incidences of individual activity at the site were recorded on all days during 2005 and 2006, when the weather was suitable for damselfly activity. Particular attention was paid to the identities of mating pairs of individuals, with their behavior noted as copulating, ovipositing, or in tandem (individuals that were paired but not copulating or ovipositing). Identifications were made directly by eye where individuals were accessible at the pond margins or otherwise using close-focusing
binoculars. The day of maturity for each individual (used for several analyses) was defined as the day on which an animal was first observed as an adult (i.e., the day that an individual was first captured and marked). No attempt was made to capture and mark tenerals (immature individuals) in this study, as they are easily damaged.

**DNA extraction and genotyping**

Genomic DNA was extracted from legs using a high-salt protocol (Aljanabi and Martinez 1997), and all individuals were genotyped at 12 microsatellite loci (LIST4-001, LIST4-002, LIST4-030, LIST4-034, LIST4-031, LIST4-034, LIST4-066, LIST4-067, LIST21-04, LIST21-006, LIST21-007, LIST21-008, LIST21-009, LIST21-010) described by Watts et al. (2004a, b) and Lowe et al. (2007). Approximately 5 ng of DNA was used for polymerase chain reaction (PCR) in a 10-µL final reaction volume containing 75 mmol/L Tris-HCl pH 8.9, 20 mmol/L (NH4)2SO4, 0.01% volume/volume Tween-20, 0.2 mmol/L each dNTP, 3.0 mmol/L MgCl2, 2 pmol forward primer, 3 pmol reverse primer and 0.25 U Taq polymerase (ABgene). Thermal cycling conditions for the primers are described by Watts et al. (2004a, b) and Lowe et al. (2007). The PCR products were pooled into one of two genotyping panels, determined by allelic size range and the 5' fluorescent dye, along with a 500 base pair size standard (GS500 LIZ, Applied Biosystems, Warrington, UK) and separated by capillary electrophoresis through a denaturing polymer on an ABI3130xl genetic analyzer (Applied Biosystems). Allele sizes were determined using the cubic model of analysis in GENEMAPPER software (Applied Biosystems).

As parentage assignment is sensitive to genotyping error, particularly when it is unrecognized, we genotyped every sample at least twice. Samples whose genotype differed between the two replicates were re-genotyped up to twice more. The mean error rate across all loci was 0.18% (range 0.0–0.24%). In >99% of cases where error occurred, mismatches were a result of allele dropouts in one PCR/genotyping round. No pattern of null alleles or large allele dropouts was detected in the final genotype data (data assessed using MICRO-CHECKER; Van Oosterhout et al. 2004).

**Data analysis**

Parentage of those individuals that reached maturity in 2006 was determined using CERVUS version 3.0 (Kalinowski et al. 2007), which generates critical log-likelihood (LOD) scores to assess parentage at a given level of statistical confidence using simulations based on user-defined input parameters. The analysis followed methods of Marshall et al. (1998). Briefly, in our pre-analysis parentage simulations (to test the resolving power of the genetic data set) we assumed that 99% of the parental generation were present in the database (actual numbers of males and females occurring during 2005 were 263 and 162 individuals, respectively), a liberal average genotyping error rate of 1% and an expected offspring population size of 611 individuals (the actual number of offspring caught in 2006) were applied. Parentage assignments were conducted using the same parameters and allocations made at the 80% and 95% confidence levels. Assignments were assessed based on critical Δ values and a required minimum LOD score of 3.0 (Slate et al. 2000); further, parental assignments were validated against field observations of adult life span. Nominal parental-pair allocations were rejected if field observations indicated that the life spans of the proposed mother and father did not overlap (with a mismatch of ±2 days to allow for error in field observations).

Heritability and variance components of maturation time and hind wing length (a proxy for adult body size) were estimated using univariate restricted-estimate maximum likelihood procedures implemented in the ASREML version 2.0 software (VCN International, Hertfordshire, UK). An “animal model” of the general form \( y = Xb + Za + e \) (where \( y \) was a vector of phenotypic values, \( b \) and \( a \) were vectors of fixed and random effects, \( e \) was a vector of residual values, and \( X \) and \( Z \) were the corresponding design matrices) was fitted to partition variance in the target phenotype to its components of additive genetic value and other random and fixed effects. For both traits, maternal identity was fitted as a random effect to account for potential maternal effects. Where maternal effect was not significantly different from zero, the analysis was rerun without this effect. For hind wing length, day of maturity was fitted as a fixed effect as adult body size is correlated with time in the reproductive season. The phenotypic variance \( (V_P) \) of a trait is given by \( V_P = V_A + V_M + V_R \), where \( V_A \) is the additive genetic variance, \( V_M \) is the maternal-effect variance, and \( V_R \) is the residual variance. Narrow-sense heritability \( h^2 \) (the similarity between parent and offspring values), was calculated as \( h^2 = V_A/V_P \). Similarly, the maternal effect was quantified as \( m = V_M/V_P \). Significance of \( h^2 \) and \( m \) were assessed via one-sample \( t \) tests using estimates provided by ASREML.

**Temporal genetic structure**

Under spatially restricted gene flow, neighboring individuals or populations are genetically more similar than distantly separated individuals/populations (Watts et al. 2007), a pattern termed isolation by distance (IBD) by Wright (1943). For organisms that reproduce across a relatively long breeding season, “temporal separation” (i.e., the difference in date of reproduction between individuals) may in effect replace spatial distance, and dispersal (gene flow) can be thought of as the difference between parent and offspring breeding times. When offspring breed at a range of different times, genetic homogeneity is maintained across the population; by contrast, if the timing of (limited) offspring reproduction is fixed, then genetic differences may accumulate
between groups of individuals that reproduce at different times during the breeding season.

To quantify the occurrence of any temporal genetic structure, two analyses were conducted. First, we determined the variation in kinship ($F_{ij}$; Loiselle et al. 1995) between pairs of individuals whose first day of maturity (the date that a mature individual was first observed and marked at the breeding site) occurred within certain time periods (i.e., 1, 2, 3, etc. days) of each other; this is analogous to spatial autocorrelation, but here temporal separation replaces spatial distance between individuals as the defining category. A Mantel test (1000 permutations) was used to assess the significance of any correlation between $F_{ij}$ and difference in day of maturity using SPAGEDI (Hardy and Vekemans 2002). This analysis was made for the population in 2005 and 2006. Second, variance in the day of maturity within full-sib and half-sib families and unrelated groups of individuals was quantified. Parentage assignments were used to designate groups of (1) full-sib, (2) maternal half-sib, and (3) paternal half-sib families, and (4) an “unrelated” category created by randomly assigning individuals to a set of “families” with a family size distribution matching that of the full-sib distribution (data not shown). For each “family” within these four relatedness classes, the variance in day of maturity was calculated. Clearly, for full-sib families consisting of only a single individual ($n = 273$), no calculation of variance is possible and these data were removed from this analysis. A Kruskal-Wallis test was used to determine whether the variance in timing of maturity differed between these categories.

**Development time**

In addition to analyzing patterns of individual maturation, development time was estimated by combining behavioral observations with the parental assignments. Where a parentage allocation for an offspring corresponded to an observation of mating behavior for that parental pair in 2005, the date on which the offspring was laid could be fixed. The period between the laying date and the day of maturity of the offspring in 2006 gave an estimate (days) of the development time to maturity. Note this estimate, therefore, includes the period of embryonic and larval development and adult maturation time. Note further, we define this estimate as development time rather than time to maturity to avoid confusion with the term “day of maturity.” Variance in development time within relatedness classes was examined in an analysis that paralleled the comparison of variance in first day of maturity. Differences between males, and gynomorph and andromorph females were assessed using a one-way ANOVA and Tukey’s post hoc multiple comparison test where appropriate.

**Results**

In total 431 and 611 individuals were observed, caught, and genotyped in the 2005 and 2006 field seasons, respectively. The first mature individual was recorded on the 20 May and the last individual reached maturity on 28 July in 2005; in 2006, these dates were 2 June and 28 July (Fig. 1). The later start of the 2006 season was due to an extended period of poor weather (see Gillingham et al. 2007). In 2005, 50% of the population had reached maturity by ordinal day 168 (36 days after the first mature individual was recorded; January 1 is day 1). In 2006 the equivalent date was ordinal day 179 (27 days after the start of the reproductive season). Notably, both seasons ended at approximately the same time (ordinal days 207 and 209).

**Temporal variation in kinship**

The relationship between pairwise kinship ($F_{ij}$) and pairwise temporal distance (difference between day of maturity) was assessed for the population in both study years (Fig. 2). In 2005 there was a qualitative, but not significant, decline of temporal genetic structure (Fig. 2a). In 2006, there was a significant decrease in average pairwise kinship ($F_{ij}$) with increasing temporal separation in date of first maturity (Fig. 2b; $r_{ss} = 40.34$, $P = 0.003$). Kinship coefficients ($F_{ij}$) were significantly ($P < 0.05$) positive from 0 up to 7 days of separation (i.e., significantly higher than the average kinship across the whole population). While average kinship was significantly different from zero, the actual values were low, suggesting that on any particular date a substantial proportion of unrelated individuals reached maturity. Beyond 30 days or more, average $F_{ij}$ became significantly less than zero (i.e., significantly lower than the average kinship across the whole population).

**Parentage assignment**

Parental allocation simulations predicted 100% (611 individuals) and 83% (509 individuals) assignment rates
at the 80% and 95% confidence levels, respectively. Misassignments in simulations were 10% (61 individuals) and 3% (18 individuals) at the respective 80% and 95% confidence levels. The subsequent analysis assigned parental pairs for 454 offspring (74% at >95% confidence. Offspring partitioned into 43 full-sib families (of ≥2 individuals), 85 maternal half-sib families, and 90 paternal half-sib families; 273 individuals formed single offspring families (i.e., without full-siblings). All offspring were assigned at the 80% confidence level, but a substantial proportion (34%) of these assignments included nominal parents with nonoverlapping life spans, suggesting a degree of ambiguity in assignments made at lower confidence levels. As a result, all subsequent analyses (i.e., heritability calculations, analyses based on relatedness classes, and estimates of development time) were conducted using only the assignments made at >95% confidence.

Heritability

Estimates of $V_A$, and subsequently $h^2$, were low and not significantly different from 0 ($P > 0.05$) for both day of maturity and hind wing length (Table 1). There was no significant maternal effect on hind wing length and subsequent removal of maternal identity as a random factor in the model did not affect estimates of additive genetic variance and heritability for this trait. A large and significant maternal effect did occur for day of maturity ($m = 0.49$; Table 1).

Development time

Development times, for the 158 individuals for which data were available, were normally distributed about a mean estimate of 371 days (SD = 9.4; Fig. 3a); maximum and minimum development times were 396 and 347 days, respectively. Development time did not significantly differ between gynomorph and andromorph females, but was significantly longer for females than for males (female = 375.4 days; male = 367.7 days; $F_{2,155} = 12.74$, $P < 0.001$; Fig. 3b). Development time was also positively correlated with first day of maturity ($r_s = 0.473$, $P < 0.001$, $n = 158$); the development time over the first and last 10 days of the season was 357.3 ± 6.3 (mean ± SD) and 378.4 ± 9.8 days, respectively (Fig. 3c). The above relationship leads to a weak, though significant, negative correlation between date of laying and subsequent development time ($r_s = -0.231$, $P < 0.001$, $n = 158$; Fig. 3d); i.e., early-laid individuals tend to mature relatively late (up to 31 days late relative to date laid) and late individuals tend to mature relatively early (up to 37 days early relative to date laid).

Relatedness and variance in development and maturation

There was a significant difference in the variance in first day of maturity between relatedness classes ($H_3 = 20.36$, $P < 0.001$; Table 2). Median variance was smallest within full-sib families and not significantly different between half-sibs and unrelated groups suggesting that “clustering” in day of maturity was most pronounced within full-sib families. In contrast, there were no significant differences between relatedness classes in the variance in development time ($H_3 = 5.44$, $P > 0.05$; Table 2).

Table 1. Heritability estimates, maternal effects, and variance components for day of maturity and hind wing length (a proxy for body size) for Coenagrion puella from a pond in Queen Elizabeth Country Park, southern England.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Day of maturity, value ± SE</th>
<th>Hind wing length, value ± SE (mm0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>181.9 ± 0.77</td>
<td>20.1 ± 0.06</td>
</tr>
<tr>
<td>$V_A$</td>
<td>12.42 ± 12.06</td>
<td>0.12 ± 0.15</td>
</tr>
<tr>
<td>$V_M$</td>
<td>120.54 ± 24.91</td>
<td>2.43×10⁻⁸ ± 1.97×10⁻⁸</td>
</tr>
<tr>
<td>$V_R$</td>
<td>109.79 ± 12.45</td>
<td>2.44 ± 0.197</td>
</tr>
<tr>
<td>$h^2$</td>
<td>0.051 ± 0.049</td>
<td>0.046 ± 0.0593</td>
</tr>
<tr>
<td>$m$</td>
<td><strong>0.497</strong> ± 0.057</td>
<td>9.49×10⁻⁸ ± 5.68×10⁻⁷</td>
</tr>
</tbody>
</table>

Notes: Variance components ($V_A$, additive genetic variance; $V_M$, maternal variance; $V_R$, residual variance); heritability estimate ($h^2$); maternal effect ($m$). Day of maturity is defined as the ordinal day on which an individual was first observed as an adult at the pond.

** $P < 0.001$.
Fig. 3. (a) The distribution of development time (number of days to reach maturity) for 158 individuals from the 2006 population. (b) A comparison of average time to first maturity between females (andromorph and gynomorph) and males; development time was significantly shorter for males compared to females. (c) The relationship between day of season (ordinal day) and development time to first maturity, and (d) the relationship between relative maturation time and the date when the egg was laid. Day 1 is January 1. For Fig. 3d, the y-axis indicates the timing of maturity of an individual relative to the time, in the previous year, that the egg from which the individual developed was laid. For example, if an egg was laid on ordinal day 180 and developed into an individual that reached maturity on ordinal day 160, in the following year, the timing of maturation relative to the day on which the egg was laid would be −20 days (i.e., relatively early). *** \( P < 0.001 \).

### Table 2. Variance in day of maturity and development time for four relatedness classes.

<table>
<thead>
<tr>
<th>Variance, by relatedness class</th>
<th>No. families</th>
<th>Median family variance</th>
<th>( Q_1 - Q_3 )</th>
<th>Kruskal-Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>( P )</td>
</tr>
<tr>
<td>Variance in day of maturity</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Full-sib</td>
<td>43</td>
<td>32.0</td>
<td>65.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal half-sib</td>
<td>85</td>
<td>91.3</td>
<td>118.8</td>
<td>NS</td>
</tr>
<tr>
<td>Paternal half-sib</td>
<td>90</td>
<td>99.6</td>
<td>139.8</td>
<td>NS</td>
</tr>
<tr>
<td>Unrelated</td>
<td>43</td>
<td>79.6</td>
<td>93.8</td>
<td>NS</td>
</tr>
<tr>
<td>Variance in development time</td>
<td></td>
<td></td>
<td></td>
<td>0.142</td>
</tr>
<tr>
<td>Full-sib</td>
<td>28</td>
<td>40.5</td>
<td>106.9</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal half-sib</td>
<td>32</td>
<td>45.0</td>
<td>53.7</td>
<td>NS</td>
</tr>
<tr>
<td>Paternal half-sib</td>
<td>40</td>
<td>42.6</td>
<td>76.9</td>
<td>NS</td>
</tr>
<tr>
<td>Unrelated</td>
<td>28</td>
<td>81.3</td>
<td>105.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Note:** For the Kruskal-Wallis ranks test, nonsignificant differences \( (P > 0.05) \) are denoted by NS. \( Q_1 - Q_3 \) is the interquartile range giving a measure of the statistical dispersion of the data.
Adult size, development time, and day of maturity

For males and females, there was a significant linear decrease in hind wing length during the reproductive season in both the 2005 and 2006 seasons (Fig. 4a, b). In both years, mean wing lengths were larger in females than males. For males in 2006, there was a significant (though weak) linear decrease in hind wing length associated with increased development time ($F_{1,96} = 13.93, P < 0.001, R^2 = 0.13$; Fig. 4c); however, the same trend was not evident for females ($F_{1,57} = 0.39, P > 0.05$; Fig. 4c).

**Discussion**

Development time is an important life history parameter. The extent to which individuals may adjust development in response to seasonal and environmental cues, and how development time and growth rate interact to shape adult body size are critical fitness components (De Block and Stoks 2003). Further, the degree of plasticity in development times and whether development is strongly genetically determined may have important consequences for population structure and long-term patterns of evolutionary change (Hendry and Day 2005, Maes et al. 2006). There is now an extensive body of literature addressing the numerous components of complex life history strategies (Johansson and Rowe 1999); many of the predictions from theoretical optimality models (e.g., Abrams et al. 1996) are now being examined using experimental approaches to directly test the responses, degree of plasticity, and interactions between life history traits such as development time and growth rate (e.g., De Block and Stoks 2003). While laboratory-based experimental studies are clearly vital, such approaches must be complemented by field-based observations to place life history responses in an ecological context and to validate predictions made from laboratory studies. However, it is not possible to track individuals from egg through larval instars to adult emergence, and traditionally this mode of development has represented a substantial problem for the study of life history parameters, and ultimately fitness, in a wide range of organisms. Here using a combination of behavioral observations and molecular data we have made field-based estimates of development time in *C. puella*. As far as we are aware, these are the first individual-based estimates of development time to be made for any aquatic insect population. They provide support for the experimental work that followed Johansson and Rowe’s (1999) initial study.

**Development time during the reproductive season**

Development time for *C. puella* varied between 347 and 396 days; notably, development time was significantly shorter in males compared to females (Fig. 3), a well-documented occurrence in odonates (Corbet 2004). Differences in mean development time between males and females (7.6 days) for *C. puella* estimated here are comparable to estimates made for related species. For
example, for *Ischnura elegans* reared in the laboratory, development time (egg laid to adult emergence) was 6 days shorter in males than females (Abbott and Svensson 2005). Abbott and Svensson (2005) also indicated that different female color morphs developed at different rates in the laboratory and emerged at different times in wild populations, though for *C. puella* studied here no difference in development time between female morphs was detected (see also Thompson 1989a).

Development time was positively correlated with maturation date (Fig. 3c), or viewed from the other end of the life cycle, development time was negatively correlated with laying date (Fig. 3d). As a result of this trend, individuals laid early in 2005 (e.g., ordinal day 150–160) reached maturity relatively late in the subsequent year and late-laid individuals reached maturity relatively early (Fig. 3d). This pattern has two interesting consequences. First it provides a mechanism by which seasonal timing of emergence and reproductive timing may be adjusted, and second it suggests that there is limited pressure for the maintenance of specific relative reproductive timing.

Life history timing in temperate odonates is primarily concerned with seasonal regulation; maximization of size at emergence, for example, is irrelevant if maturity occurs when environmental conditions are unsuitable for reproductive activity (Johansson and Rowe 1999). The pattern of decreasing development times during the reproductive season observed here is one mechanism by which seasonal reproductive timing may be maintained; if a parent matures late, its subsequently late-hatched offspring are likely to have a short development time and thus will mature relatively early. Likewise if a parent matures early, its offspring are likely to develop slowly and subsequently mature relatively late. The acceleration of development rates in response to late season cues are well established from laboratory studies (e.g., Plaistow and Siva-Jothy 1999, Johansson et al. 2001, De Block and Stoks 2004); photoperiod in particular provides temporal cues, and a decrease in development time is a common mechanism by which odonate larvae advance the relative timing of their life cycles (Johansson and Rowe 1999, De Block et al. 2008).

In this study, the timing of maturity relative to the point at which an individual was laid was advanced in the most extreme case by up to 37 days (Fig. 3c, d). De Block et al. (2008) showed the life history response to time stress in *Lestes eurinus* (10–12 months larval stage) was a difference in development time close to 35 days. Whether such rapid development incurs costs to reproductive potential or is associated with increased mortality risk is difficult to assess. Certainly theoretical life history models (Abrams et al. 1996) and experimental studies suggest that the maintenance of adult body size, for example by increased foraging, increases predation risk (Johansson et al. 2001, Stoks et al. 2005). The interaction between growth rate and development time, therefore, is likely to be critical in determining the pressures influencing developmental plasticity (see Discussion: Development time and seasonal decline...). Regardless of the potential costs of accelerating development, the overriding benefit is the synchronization between life cycle transitions and season. The development pattern described here drives individ-

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**PLATE 1.** A pair of *Coenagrion puella* (Odonata: Zygoptera) in a mating wheel. Damselflies (e.g. male K00 and female F60, shown here) were marked and observed at the Queen Elizabeth Country Park, United Kingdom, during the 2006 field season. Note that the middle leg on the left side of each animal has been removed for the purpose of DNA extraction (see Methods). Photo credit: P. C. Watts.
uals toward a midpoint in the reproductive season. This response should generate the greatest density of emerging/maturing individuals mid-season, a pattern that was observed in both study years for the C. puella population at QECF (Fig. 1).

However, other studies have suggested that relative reproductive timing may be an important component of fitness and that there may be selection for early reproductive timing in some cases (Plaistow and Siva-Jothy 1999). For example, early maturation, and consequently increased breeding time, may be advantageous in species where the reproductive season is short (Johansson and Rowe 1999): early-hatching larvae have more time to develop before the onset of winter and will be larger relative to later emerging individuals. Subsequently, smaller individuals are disadvantaged by intraspecific competition for food and increased risk of cannibalism (McPeek and Crowley 1987, Gribbin and Thompson 1990, Anholt 1994). The developmental timing of early-hatching larvae is less commonly addressed though clearly time constraints still occur (i.e., short development times for early-hatched larvae may result in emergence too early in the season). Here long development times for the earliest laid individuals suggest limited pressure for the maintenance of early emergence and subsequent reproduction. Rather it seems likely, given the evident degree of developmental plasticity, that development time may interact with other life history traits (e.g., growth rate) to optimize other physical traits (e.g., size).

Development time and seasonal decline in adult size

A decline in body size occurred for males and females in both seasons and was consistent across both study years (Fig. 4a, b). The relationship between development time and size differed between males and females. For males, size decreased with increasing development time; for females there was no pattern of size variation with development (Fig. 4c). Sex specific differences in optimal life history responses have been reported extensively. Although large size is advantageous in both sexes, the optimum size for females with respect to lifetime egg production is larger than the optimum size for males with regard to lifetime mating success (Thompson 1989b, Thompson and Fincke 2002, De Block and Stoks 2003). Thus, age at emergence/maturity is likely to be more important for males, whereas size at maturity is likely to be more important for females (see review by Morbey and Ydenberg 2001). The lack of a relationship between size and development time for females reported here certainly supports this notion; size appears to be maintained (presumably as a result of compensation by variation in growth rate) independently of development.

The decrease in size associated with increased development time for C. puella males, is at first glance surprising although it has been reported in controlled laboratory studies (Lawton et al. 1980, Pickup and Thompson 1990). While the relationship between size and development time has remained unclear for odonates (Abrams et al. 1996), there are generalities. For example, De Block and Stoks (2005) indicated that late-hatching L. viridis larvae displayed decreased development times, increased growth rates, and higher body mass in comparison to early-hatched individuals. In L. congener, Johansson and Rowe (1999) described a similar pattern and interpreted this as a trade-off between size and timing of emergence. For C. puella males our field estimates suggest that development time and growth rate both respond to seasonal position, as the shortest development times were associated with largest body sizes (Fig. 4c). As a result, individuals laid relatively late tended to accelerate development and growth and subsequently emerged relatively early as large adults. This observation from the current study and previous observations (e.g., Johansson and Rowe 1999, De Block and Stoks 2004, Strobbe and Stoks 2004) provides an important insight: there is not necessarily a trade-off between age and size at maturity, as is traditionally assumed (Abrams et al. 1996). Late-hatching larvae may decrease development times and maintain (or even increase) size at maturity, by increasing growth rate. That longer development times may be associated with smaller adult body size has been noted previously; optimality models, supported by some empirical studies, suggest that rapid growth mediated by increased feeding activity may result in increased mortality via predation. Thus, for early-hatched larvae, with a relatively long period in which to develop, there is little pressure for maximizing growth rate and it may behoove individuals to adopt a slower growth strategy with decreased mortality risk. Clearly, we are unable to assess growth rate from our field observations; though the size vs. development relationships presented here suggest that growth rates are likely to be plastic.

Our field observation of development time and adult body size supports many of the predictions made from laboratory studies. However, a note of caution should be made. The most detailed experimental studies of life history responses in odonates have concerned members of the genus Lestes. Lestids overwinter as obligately diapausing eggs, with rapid larval development and adult emergence ~3 months after egg hatching; this developmental pattern makes them particularly amenable to experimental studies (by reducing the period over which larvae have to be maintained in a laboratory) but is atypical of odonates (Corbet 2004). Comparisons between lestids and coenagrionids, i.e., odonates with differing life cycle strategies, must as a result be made with caution. Nevertheless, our observations imply that some of the physiological and developmental responses described in the laboratory are general and manifest in wild odonate populations.

Kinship and temporal separation

The extent of temporal variation in reproduction, both in parents and their offspring, clearly determines
the degree of temporal genetic structure during a reproductive season. While there are no direct estimates of the extent to which life cycle timing is genetically determined in odonates, there is some indirect support for a genetic component to timing. For example, maternal color morph affected male and female offspring development time in I. elegans (Abbott and Svensson 2005); De Block and Stoks (2005) determined growth rate differences between early- and late-hatching larvae in common garden experiments suggesting a genetic difference associated with hatching time. For C. puella here, the estimate of $h^2$ for first day of maturity was low (Table 1), indicating that parent and offspring reproductive times were uncorrelated and that there is unlikely to be a strong genetic component to life cycle timing. Given the degree of variation in development and the pattern of changing development time during the reproductive season, a low estimate for $h^2$ of reproductive timing is unsurprising. Nevertheless, despite developmental variation and limited heritability for reproductive time, a qualitative pattern of temporal genetic structure was evident in the population (Fig. 2a, b).

This weak pattern of temporal genetic structure appears to be a result of a degree of consistency between full siblings in reproductive timing. While heritability for reproductive timing was weak, i.e., parents did not produce offspring with relative reproductive times similar to their own, timing of maturity was more similar between full siblings than between half siblings and unrelated groups (Table 2). We suggest that the weak clustering of relatives is a simple result of full siblings being laid at the same time. Females of C. puella mate once per day and then lay all of the eggs that they have produced (Banks and Thompson 1987). As a result full siblings are laid on the same day, likely as a single egg clutch. Given the same laying date and some consistency in development time (as suggested by the pattern of change in development time during the season), a degree of clustering in the subsequent timing of maturation is likely. Half-sib offspring on the other hand are laid, at the closest interval, on consecutive days and up to several weeks apart (depending on the adult life span of the mother) and as a result are likely to be less clustered in terms of timing of maturity in the subsequent reproductive season. The temporal clustering of related individuals as a result of clutch laying is further supported by the maternal effect on timing of maturity ($m = 0.497$; Table 1). This large effect indicates that maternal identity, independent of the maternal trait value, had a large influence on the timing of maturity of offspring. A combination of limited adult life span relative to the total length of the reproductive season (such that a particular mother can only produce offspring for a part of the reproductive season), consistency of development time, and the laying of eggs in clutches potentially produces this effect: mean trait values for the offspring of any particular mother are likely to differ from the mean trait value for the population as a whole resulting in a maternal effect. Variation in maternal investment per egg between females occurring at different points in the season might also produce this effect although it would seem unlikely given that late laid eggs, for which we might speculate a reduced maternal investment, go on to be the fastest developing and largest individuals in the subsequent generation.

The approach used in this study, combined direct and molecular tracking of individuals and their offspring, offers the unprecedented opportunity to explore many other life history and other traits (including fitness, the “Holy Grail” of behavioral ecology) in both odonates and other organisms with complex life histories.

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**Literature Cited**


