Odonatologica 25(3): 261-273 *September 1, 1996*

CAPTIVE BREEDING OF *ISCHNURA ELEGANS* (VANDER LINDEN): OBSERVATIONS ON LONGEVITY, COPULATION AND OVIPOSITION (ZYGOPTERA: COENAGRIONIDAE)*

G. COOPER[1], P.W.H. HOLLAND[2] and P.L. MILLER
Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom

Received June 20, 1995 / Revised and Accepted January 6, 1996

The procedures used to obtain laboratory mating and oviposition of *I. elegans*, involving both individuals raised from larvae in captivity and others captured from the wild as imagines, are reported. Egg-clutches laid by 25 ♀ of known mating histories and 26 ♀ caught from the wild as adults are described. Observations relating to survival in captivity, intra-specific predation, age at first copulation, copulation duration, oviposition and ♀ inter-copulation interval are reported and discussed. Mean copulation duration was greater for non-virgin ♀♀ than for virgins (average durations 104 and 78 min, resp.). The number of days a ♀ survived after her first copulation was the best predictor of her life-time egg production. This fecundity was not affected by the number of times a ♀ copulated. ♀♀ were seen to repel many mating attempts. Of 33 ♀ which mated in captivity, 15 mated more than once, with more than half of these rematings occurring within 4 days of the previous copulation, after fertile eggs had been laid. This demonstrates that at least some ♀♀ are willing to remate soon after successful sperm transfer by a previous ♂, apparently contradicting a previous description of this sp. as monogamous in England.

INTRODUCTION

Laboratory breeding of a zygopteran species was initially reported for *Ischnura verticalis* by GRIEVE (1937) and for *I. elegans* by KRIEGER & KRIEGER-LOIBL

[1] **Corresponding Author and present address**: Department of Genetics, University of Cambridge, Downing Site, Cambridge, CB2 3EH, United Kingdom
[2] **Present address**: School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, United Kingdom
* Peter Miller died on 24 March 1996. This paper is dedicated to Peter: a wonderful colleague and friend, and an inspiration to all of his students.

(1958). JOHNSON (1965, 1966) published details of his procedures for breeding *I. damula* and *I. demorsa*. A similar method was used by CORDERO (1990a) for *I. graellsii* and he reports matings in his insectaries by several other zygopterans – *I. elegans, I. pumilio, Ceriagrion tenellum, Lestes viridis, L. virens, L. barbarus* and *L. dryas*. Compared to some other odonates, ischnurans appear to be relatively easy to breed in the laboratory as the larvae do not require running water, there is no eleborate courtship, and females oviposit alone, into vegetation at the water surface.

Species of the genus *Ischnura* copulate for longer than other odonates, but generally do not exhibit post-copulatory mate guarding. The ability of females to repel unwanted approaches from males and observations of natural populations of *I. elegans* in England suggest that females only mate once per lifetime (PARR & PALMER, 1971). However, in southern France most females mate more than once (MILLER, 1987a), providing an opportunity for sperm competition. That sperm competition is important in this species is supported by the observation that copulating males remove some of the sperm stored by the female from previous matings (MILLER, 1987a, 1987b).

METHODS

COLLECTION AND MAINTENANCE OF LARVAE. – Larvae were collected between April and June from several sites close to Oxford where adult *I. elegans* were known to occur. Collections were made at Wychwood Forest (O.S. Ref. SP 338169) and Kennington Pond (SP 520 033), in 1991, and from Vauxhall Gravel Pit (SP 399 058) in 1992. Zygopteran larvae were found to be at highest density amongst the debris at the foot of water-vegetation. Debris and larvae were scooped from the water and stored in plastic bags for up to an hour during transport to the laboratory. Species identification was carried out on a few larvae (G. Vick *in* MILLER, 1987c) to confirm that the majority of the larvae collected were *I. elegans*, but no attempt was made to remove the other species (mostly *Enallagma cyathigerum*). These non-ischnurans were released into the wild on emergence.

Larvae were kept in a 45 × 35 × 35 cm glass aquarium, two-thirds full of water (tap-water which had been left to stand for several hours) with constant aeration. Aquatic vegetation was provided, and larvae were fed with live *Tubifix* worms and *Daphnia*. Twigs were provided as supports for emerging damselflies. The aquarium was contained within a perspex box of side 50 cm, to prevent the escape of teneral adults. A small number of larvae were kept at 5°C, for up to 10 weeks, in loosely covered plastic boxes, containing water and some pond-weed, to delay emergence.

One cause of mortality shortly after emergence was for the teneral to fall back into the water. Several newly-hatched damselflies were observed to have severe distortions of the abdomen, and these individuals did not survive more than 24 hours. Wing damage also occurred, either due to incomplete expansion of areas of the wings or mechanical damage sustained by the newly inflated wings. To minimise this damage, tenerals were not handled until they were flying frequently. Individual identification codes were written on the left hind-wing with a waterproof marker pen, before transferring tenerals to cages described below.

COLLECTION AND MAINTENANCE OF ADULTS. – Adult *I. elegans* were collected from the same sites as larvae and also from the River Cherwell (O.S. Ref. SP 522 070). Individuals were placed in empty film canisters (diameter 3 cm, height 5 cm) containing grass and kept in the shade and transported to the laboratory as soon as possible (< 1 hour). Any pairs that were captured in copula were placed in a box (17 × 11 × 5.5 cm), where they were left until copulation had ceased. In the

laboratory, individuals were labelled for later identification and kept as described below.

Adult *I. elegans* were kept in cuboid cages of size varying between 17 and 30 cm. Cages consisted of wooden frames covered in nylon or cotton mesh (weave size 1-3 mm), with overlapping mesh secured with Velcro to allow access. The vertical sides of the cages were covered with aluminium catering foil to reflect light into the cages from overhead lighting and to minimise disturbance (JOHNSON, 1965). Twigs were placed in each cage to provide perch-sites, although the damselflies also perched on the mesh walls of the cage. Cages were kept in a small laboratory (temperature range 18-32°C, average daily maximum 24°C). Additional heat and light was provided during the day by means of a desk-lamp.

Adult damselflies were fed from cultures of *Drosophila*. To prevent *Ischnura* from getting stuck in the *Drosophila* food medium, the open culture bottles were covered with Parafilm, in which holes were punctured which were large enough to allow free movement of *Drosophila*, but prevented the entry of damselflies. To increase humidity and provide drinking water, beakers of water were provided which were covered with perforated aluminium foil, such that water was accessible at a small depth with good footholds to prevent drowning, but without providing an attractive oviposition site. Adults were periodically observed to perch on the foil and take water through the mouth.

OBTAINING LABORATORY MATINGS OF *I. ELEGANS*. – Opportunities for mating were provided by introducing one or more males into a cage containing up to three females. Close observation appeared to have a disruptive effect on mating behaviour, causing the *Ischnura* to move away from the observer and hide behind twigs or show erratic escape reactions, flying into the sides of the cage. To avoid disturbance, observation was kept to the minimum required to assess the identity of individuals involved in copulations and the duration of matings.

Cages were checked at maximum intervals of 10 minutes. Any mating behaviour was noted, and pairs seen to be in tandem were observed continuously until either the 'wheel position' was taken up, or the pair separated without copulation. Mating pairs were observed at 5-minute intervals until copulation was complete and the pair separated. Males which made attempts to mate but were rejected by the first female(s) with which they were placed, were removed from the cage after up to two hours and placed with other female(s) with whom they usually attempted to mate, sometimes successfully.

Several attempts at hand-pairing were made, by holding a pair of damselflies and bringing the male's abdominal appendages into contact with the female's prothorax, as described for *Calopteryx* species by OPPENHEIMER & WAAGE (1987). These attempts were not successful, as the male *I. elegans* did not open their claspers to grasp presented females.

OVIPOSITION. – Females which had mated in captivity, or were wild-caught and did not appear to be teneral, were provided with chromatography paper (Whatman 3MM) with one end in a 90 mm petri dish filled with water to a depth of about 5 mm. The paper was labelled, in pencil, with the identity of the female and the date, before placing in the water, where it was accepted by females as an oviposition substrate. On two occasions, females appeared to be ovipositing in the mesh of the cages and in the Parafilm over the *Drosophila* cultures. The females concerned were placed on the damp filter paper where they continued to oviposit. Fewer than 12 eggs were detected in each of these alternative oviposition sites. Each female that had mated in captivity or could have mated in the wild was kept in a separate cage so that eggs collected were of known maternity.

Filter papers were removed from the cages of mated females daily, between 09:15 h and 09:45 h. These were maintained in water-filled petri dishes, at room temperature. After two days, by which time fertile eggs had darkened, the papers were examined and those papers where no eggs were visible were discarded. The remaining papers were rinsed to remove any adhering *Drosophila* and placed in fresh water. Fertile eggs were counted between two and ten days after oviposition. Infertile eggs, which did not darken in colour, were not easy to see in the filter paper, so these were only counted when they comprised the entire clutch of eggs. To facilitate the rapid counting of eggs, each filter paper was laid flat on cling-film over a light source.

SURVIVAL IN CAPTIVITY

The survival times for *Ischnura elegans* adults emerging in the laboratory, or captured as tenerals, are shown in Figure 1. Individuals which died during emergence or did not survive more than one hour post-emergence are not included in this figure, or in calculations of averages. The mean longevity for males is 4.4 days (n=67; maximum 10 days), compared to 10.4 days for females (n=67; maximum 36 days). Mean survival times for individuals captured from the wild as non-teneral adults were 2.6 days (n=63; maximum 10 days) for males and 8.5 days (n=35; maximum 34 days) for females.

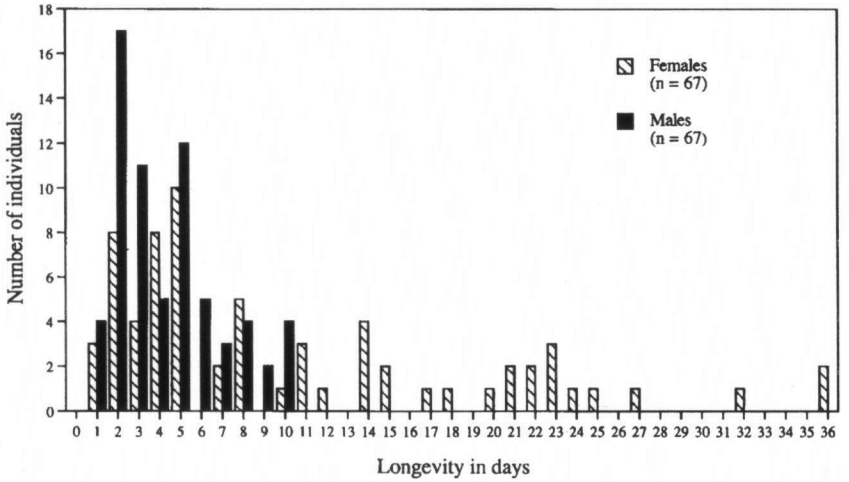

Fig. 1. Longevity of adult *Ischnura elegans* after emergence in captivity.

On sixteen recorded occasions, adult *I. elegans* were killed, and in at least nine cases partially eaten, by others. There was only one case of a male killing another male and one of a male killing a female. However, there were four cases of a female killing another female and ten instances in which females killed and consumed the males that had been introduced to their cages for mating. One notable female killed a male and was seen to be eating him, and less than three hours later killed and ate parts of another female, despite the presence of large numbers of *Drosophila*.

COPULATORY BEHAVIOUR

The minimum age at which copulation occurred in this study was 3 days for males (in 2 cases out of the 14 in which male age was known) and 5 days for females (in 6 cases out of 25), although many individuals were given the opportunity to mate earlier than this. The range of ages at first copulation was 3-8 days for

males and 5-13 days for females. Females which had emerged in captivity survived for an average of 13.3±1.6 days (±s.e.; n=25) after their first copulation.

A range of behaviour was seen amongst mature adults placed together for breeding. Some males made active and persistent attempts to mate, but some only made one attempt and others made no observed approaches to females. Those males which attempted actively to mate did not appear to discriminate between mature females and those less than five days old.

Females also exhibited a variety of patterns of behaviour. Some appeared to hide from the males by moving around their perches, some vigorously resisted male attempts to mate and some even behaved aggressively by flying directly at the males, in some cases killing them by biting the thorax or abdomen. Still other females offered no apparent resistance to male mating attempts, and copulation ensued. Females less than five days old always resisted mating attempts as described below.

Females that were not willing to mate appeared to resist copulations by flying away from approaching males. If a male succeeded in grabbing a female with his legs a struggle ensued, during which the female waved her abdomen and beat her wings, apparently trying to escape from the male's grasp. This often resulted in the male flying away without having attained the tandem position. Whilst in the tandem position, several females were observed to rub their fore-legs over their heads, apparently pushing at the males abdomen. This behaviour was seen in two females who refused to form the wheel position and in one female who had been held in the tandem position for at least nine minutes after the wheel position was broken following copulation (typically tandem was maintained for just two to three minutes post-copulation). Similar head-grooming in females held in tandem and resisting copulation is reported by KRIEGER & KRIEGER-LOIBL (1958).

Typically it took 3-4 minutes from the time of tandem-formation for a pair to reach the wheel position. Where females did not co-operate, males persisted in their attempts for 4-7 minutes. In one instance, however, a pair was in tandem for 47 minutes, making repeated attempts to reach the wheel position, which failed even though there seemed to be female co-operation. The pair occasionally appeared to have attained the wheel position, only for it to break open again within 5 minutes. Neither individual was involved in any other copulation attempt and the reason for the failure to mate was not ascertained, although genital damage is a possible explanation.

The time for which the wheel position was maintained was recorded as the copulation duration. The average copulation duration was 86.8±4.7 minutes (±s.e.; n=70; range 25-191 minutes). Copulations involving virgin females (mean ± s.e. = 77.8±5.4 minutes; n=25) were significantly (2-tailed t-test, t=2.16; 35 df; $p<0.05$) shorter than those involving females who had mated once or twice previously (103.7±8.7; n=12). Second copulations were an average of 33 minutes longer than first copulations – a significant difference (paired t-test, t=2.29; 11df; $p<0.05$).

It could be suggested that the longer copulations of non-virgin females were an effect of female age on copulation duration, as age and female copulation number are clearly not independent (females must be older at the time of second mating than at their first mating). To investigate any effect of female age on copulation duration, the data-set was divided into groups according to female copulation number. For each of the groups no significant correlation between female age and copulation duration was detected. (Probabilities were derived from the correlation coefficient, r, using table 7 of FISHER & YATES, 1963. No previous copulations: r^2=0.0018; n=25; NS (not significant at 5% level). One previous copulation: r^2=0.019; n=12; NS. One or two previous copulations: r^2=0.15; n=14; NS).

Details of age and mating history were only known for a small proportion of males, but amongst these there was no correlation between copulation duration and either male age (r^2=0.030; n=14; NS) or number of previous copulations (r^2=0.25; n=14; NS).

OVIPOSITION

Oviposition often began as little as 30 minutes after the end of copulation. Most oviposition occurred between 12:00 and 19:00 h, although it was occasionally ob-

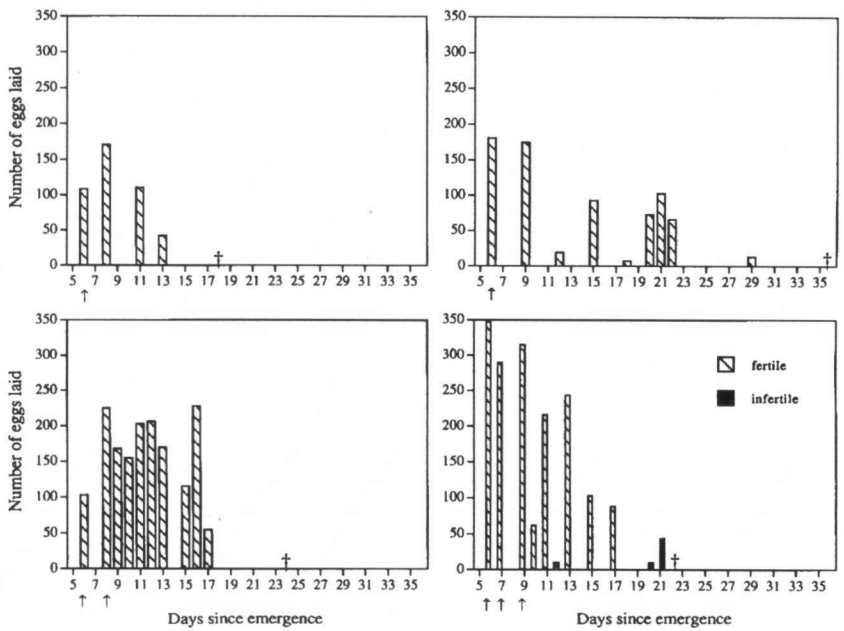

Fig. 2. Bouts of oviposition for four *Ischnura elegans* females which emerged in captivity. ↑, copulations; †, day of death.

served earlier in the day, even before 10:00 h. In 36 cases out of 50, females oviposited on the day of, or the day after, copulation and the longest interval between copulation and oviposition was six days. Six of the females which had emerged and mated in captivity did not lay any eggs after copulation, these individuals surviving for an average of 2.67 days after mating (range 0-5 days).

To illustrate the patterns of oviposition observed, the egg-clutches laid by four lab-emerged females are represented by a series of histograms in Figure 2. The patterns of clutch size for individual females is inconsistent, although of those 18 females which laid two or more clutches of eggs, 15 laid their largest clutch first. When all clutches are considered together, a significant negative correlation between clutch size and female age is apparent (r^2=0.26; 142 df; $p<0.001$). There is no significant correlation between clutch-size and the number of days since the last clutch (r^2=0.018; 121 df; NS).

The vast majority of eggs in each clutch were fertile (estimate>90%, for 113 clutches), although there were nine clutches of eggs which were entirely infertile. Three of these clutches were the only eggs laid by wild-caught females, who may never have mated. The remaining infertile clutches comprised one clutch which was the first laid by a female, three which were the last clutches laid by respective females before death, one penultimate clutch (which was followed by another infertile clutch), and just one clutch which was both preceded and followed by fertile eggs.

The average number of days elapsed between oviposition and the hatching of eggs was 12.5±0.17 (±s.e.; n=94). Hatching continued over a period of 2-3 days.

The number of eggs laid by each female was recorded. The females of known history (i.e. emerged in the laboratory or caught as tenerals) which had mated at least once, laid a mean of 742±125.3 eggs (±s.e; n=25; range = 0-2016 eggs) over their lifetime. Female longevity was expected to be an important factor in determining the lifetime egg production (BANKS & THOMPSON, 1987; CORDERO, 1990b; FINCKE, 1986, 1988; KOENIG, 1987; McVEY, 1988). When this was tested, a strong positive effect of female longevity on egg production was detected (r^2=0.47; 23 df; $p<0.001$). Female longevity was then considered as two separate additive components: age at first copulation and survival after first copulation, both measured in whole days. The female's age at first copulation showed no significant negative correlation with lifetime egg production for either singly-mated females (r^2=0.043; n=13; NS), multiply mated females (r^2=0.18; n=12; NS) or all females considered together (r^2=0.121; n=25; NS). Similarly, no significant effect of age at first copulation on lifetime egg production was detected when females were next considered in separate groups, according to the number of days they survived after the first copulation (females surviving 0-14 days; r^2=0.015; n=13; NS: females surviving 15-30 days; r^2=0.29; n=12; NS). This suggested that the number of days survived after the first copulation is the best predictor of lifetime egg production (r^2=0.58; n=25; $p<0.001$).

The number of copulations by any one female was in part influenced by when eggs were laid (females were not given an opportunity for a second copulation until they had laid at least one clutch of eggs, and most females were not given the opportunity for a third copulation). Clearly, females who did not lay eggs or who died soon after the first copulation would not have had the opportunity for remating, which would be likely to enhance artificially any positive effect of multiple matings on lifetime egg production. To control for the effects of differential survival, analysis of the relationship between number of copulations and average egg production per day (after first copulation) was carried out. No significant correlation was detected (r^2=0.052; n=25; NS). As a further test for any influence of number of copulations on number of eggs laid, females were considered in two groups according to survival times after first mating. Number of copulations had no significant effect on the total number of eggs laid (females surviving 1-14 days; r^2=0.21; n=12; NS – females surviving 15-30 days: r^2=0.024; n=12; NS), or on the average number of eggs laid per day survived after first copulation (females surviving 1-14 days: r^2=0.0046; n=12; NS – females surviving 15-30 days: r^2=0.031; n=12; NS), in either group.

FEMALE INTER-COPULATION INTERVAL

After females had laid their first clutch of eggs, they were given the opportunity to remate when suitable males were available. No female was given the opportunity to re-mate on the same day. The longest interval between copulations was 13 days and the mean inter-cop interval was 5.9±0.93 days (±s.e.; n=17). Out of 17 rematings, 9 took place within four days of the previous mating. This provides evidence that some females are willing to re-mate soon after the previous copulation even when successful sperm transfer had occurred, as demonstrated by the oviposition of fertile eggs from the first mating. This contrasts with the finding that *I. verticalis* only mates once per lifetime, unless the initial mating is interrupted so that sperm transfer is reduced (FINCKE, 1987).

DISCUSSION

SURVIVAL IN CAPTIVITY

Males of *Ischnura elegans* had shorter average longevity (4.4 days) and survival times (2.6 days) than females (10.4 and 8.5 days respectively), which may be due, at least in part, to the higher density in which males were kept. Each post-copula female was kept in isolation so that the maternity of any eggs oviposited was known, leaving just one or two cages available for males, which consequently were kept at higher densities (up to 7 per cage). This could have resulted in aggressive behaviour and competition for food causing additional physiological stress, resulting in

earlier deaths. Males were also more often the victims of intra-specific predation than females.

For wild *I. elegans* in Britain, maximum survival estimates of 42 days for males and 50 days for females have been reported (PARR, 1973), and mean length of life is estimated to lie in the range 3.3-12.4 days for males at three different colonies (insufficient data available to estimate female survival). Data from the current study show that the conditions in which the damselflies were kept were suitable to allow the survival of at least some individuals for long periods, but not for as long as has been observed in the wild. It has been suggested that insects age more rapidly at higher temperatures (see discussion in SOHAL, 1986), and higher night-time temperatures in the laboratory than those experienced by wild damselflies could partially explain shortened lifetimes in captivity.

The reasons for the intra-specific predation, which in the majority of cases involved eating portions of the victim, are not clear. CORDERO (1992) mentions one case of cannibalism observed in a study of a wild population of *I. graellsii* and FINCKE (1987) reports captive mature *I. verticalis* females eating immature females and a mature male, in addition to newly emerged individuals of both sexes. The killing could constitute predation for feeding, perhaps because *Drosophila* did not provide sufficient nutrition (especially for females), although JOHNSON (1965) did not report any cannibalism when he used adult *Drosophila* as food for *I. damula* and *I. demorsa*. Alternatively, the killings could be an aspect of defence of a perch site or, in some cases, a method of disposing of males persisting with unwanted mating attempts. There is no apparent correlation between high density and intra-specific killing, with the number of individuals per cage varying from two to six, and only two damselflies present in eight of the fifteen recorded cases.

AGE AT FIRST COPULATION

The minimum age at copulation of five days for females corresponds with the maturation period of 5-6 days reported for *I. graellsii* in captivity (CORDERO, 1990b). Males in this study did not mate until at least three days after emergence. Higher night-time temperatures and consistent food supply might be expected to make average maturation times slightly shorter in captivity than in the wild. However, PARR & PALMER (1971) reported observations, for *I. elegans* in northern England, of one male mating at three days old and one female mating at 4 days old (from several thousand individuals), although they do not indicate the typical age at first mating.

COPULATION DURATION

The mean copulation duration of 86.8±4.7 minutes (±s.e.; n=70) was shorter than values observed in the high density populations studied in Germany (KRIEGER

& KRIEGER-LOIBL, 1958) and southern France (MILLER, 1987b), which have mean durations of more than 3 hours and 5.4 hours respectively. A large proportion of this time comprises extended inactive pauses; this contrasts with the continuous copulatory activity of tethered or decapitated pairs of *I. elegans*, which has a duration of approximatley one hour (MILLER, 1987b). The extended copulations are thought to represent a form of in-copula mate-guarding to prevent take-overs by other males before a female is ready to oviposit. *I. elegans* was exceptionally abundant at the southern France study site (MILLER, 1987b), and this high density is likely to lead to greater copulation durations than in the current study, although differences in temperature may also have some effect.

TIME OF DAY. – CORDERO's (1990b) study of *Ischnura graellsii* found that the time of day at which copulation started correlated negatively with copulation duration, especially at high male density. This suggests that the longer copulations were a mate-guarding strategy to ensure that copulations terminated synchronously, giving females less opportunity to remate before ovipositing. At low male density, where the probability of a female mating twice in one day would be small, the optimal male strategy might be to shorten copulation duration so as to maximise his opportunity for a second copulatory encounter. In the current study, the time of day at which copulations started did not appear to affect copulation duration. However, it is possible that some effect might exist at high density, but was not detected due to the variability of photoperiod and temperature, the restricted timing of opportunities for mating, and the low density of males in this study.

PREVIOUS COPULATION. – If male *I. elegans* control copulation duration, as shown by MILLER (1987b), and alter their time investment according to the reproductive potential of the female, copulations with older females would be shorter than copulations with young females with greater life-expectancies. However this does not appear to be the case, as no effect of female age on copulation duration was detected when copulations involving virgin and non-virgin females were analysed separately. The observation that copulations involving females mating for a second time (103.7±8.7; n=12) were longer than those involving virgin females (77.8±5.4 minutes; n=25) could reflect the time taken for the second male to remove the stored sperm from the first copulation.

It might be expected that the time taken for sperm removal would depend on the quantity of sperm present in the sperm storage organs. This study found no correlation between copulation duration and either inter-copulation interval (r^2=0.0053; n=12; NS) or number of eggs laid since the previous copulation (r^2=0.0034; n=12; NS), suggesting that the effects of the small changes in volume of stored sperm resulting from sperm utilisation or sperm breakdown and absorption are negligible. However, variance in either male sperm removal ability, or perhaps female cooperation in sperm removal, or some other factor, could easily obscure the small changes resulting from sperm utilisation between copulations.

CORDERO (1990b) found a similar increase in copulation duration if the female

had mated previously, and suggested that males could detect the presence of sperm in the female's reproductive tract. Yet some stage I (sperm removal, see MILLER & MILLER, 1981) activity still occurs, even if the female's reproductive tract contains no sperm (i.e. when it is her first copulation). This could suggest that the male goes through some sperm removal process, for a minimum period of time, which could be stimulated to continue if the male detects that sperm remain, or signalled to stop when the male makes direct contact with the walls of the female's reproductive tract. MILLER (1987b) found that *I. elegans* copulations continue after decapitation of both members of the mating pair, with duration similar to that for tethered pairs, and that dissection of the female's reproductive tract had little effect on the duration of the different copulatory stages. He therefore concluded that sperm removal depends on a stereotyped programme of action rather than on information about the sperm content of the female's storage organs. This observation suggests that there is a 'default' period for which sperm removal activities will continue, which may be extended in response to some signal, presumably related to the presence of sperm in the female's storage organs or to some response from the female.

OVIPOSITION

In 36 cases out of 50, females oviposited eggs on the day of, or the day after copulation. Many odonates oviposit immediately after copulation, either in tandem or with non-contact guarding, but ischnuran females oviposit alone, either repelling male approaches with warning displays (KRIEGER & KRIEGER-LOIBL, 1958) or visiting oviposition sites relatively late in the day, after the peak of male activity (MILLER, 1987b). The failure of some females to oviposit on the same days as copulation may reflect a lack of mature eggs, or could possibly have been the result of conditions in captivity, such as dissatisfaction with the oviposition substrate provided. Clutch sizes tended to decrease with increasing female age, presumably reflecting a decline in the rate at which eggs were matured. In contrast to *Coenagrion puella*, in which clutch-size increases with inter-clutch interval (BANKS & THOMPSON, 1987), the current study found no significant correlation between clutch-size and the number of days since the last clutch (r^2=0.018; 121 df; NS) in *I. elegans*.

FEMALE INTER-COPULATION INTERVAL

The mean inter-copulation interval was 5.9±0.93 days (±s.e.; n=17). This interval is likely to be longer than might have been observed if males and females had free access to each other at all times, rather than artificially-controlled encounters of limited duration on only 6 days per week. Nine out of the 17 rematings took place within four days of the previous mating, showing that some females will remate soon after the previous copulation even when successful sperm transfer had

occurred, as demonstrated by the oviposition of fertile eggs.

In southern France, MILLER (1987a) found that some female *I. elegans* accepted second males on the same or the subsequent day after mating. In northern England, in contrast, PARR & PALMER (1971) observed few copulations and concluded that females generally mated only once. The current study has shown that some females mated more than once in captivity, despite their apparent ability to reject unwanted mating attempts. This observation is reinforced by genetic data (COOPER, 1994) which showed that of 9 females which laid eggs in captivity after capture from the wild, 6 had mated with at least two males. This demonstration that females of the species are not monogamous strongly suggests that sperm competition is an important factor in the evolution of mating strategies in populations of *Ischnura elegans* in England.

ACKNOWLEDGEMENTS

We thank the ICRF unit, Oxford for supplying *Drosophila*, and W. AMOS for helpful comments on the manuscript. This work was supported by a SERC studentship to G.C.

REFERENCES

BANKS, M.J. & D.J. THOMPSON, 1987. Lifetime reproductive success of females of the damselfly Coenagrion puella. *J. Anim. Ecol.* 56: 815-832.

COOPER, G., 1994. *Analysis of genetic variation and sperm competition in dragonflies*. PhD thesis, Univ. Oxford.

CORDERO, A., 1990a. The inheritance of female polymorphism in the damselfly Ischnura graellsii (Rambur) (Odonata: Coenagrionidae). *Heredity* 64: 341-346.

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

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

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

FINCKE, O.M., 1987. Female monogamy in the damselfly Ischnura verticalis Say (Zygoptera: Coenagrionidae). *Odonatologica* 16: 129-143.

FINCKE, O.M., 1988. Sources of variation in lifetime reproductive success in a nonterritorial damselfly (Odonata: Coenagrionidae). *In*: T.H. Clutton-Brock, [Ed.], Reproductive success, pp. 24-43, cumulative references pp. 487-520, Univ. Chicago Press, Chicago.

FISHER, R.A. & F. YATES, 1963. *Statistical tables for biological, agricultural and medical research* [6th edn], Oliver & Boyd, Edinburgh.

GRIEVE, E.G., 1937. Studies on the biology of the damselfly, Ischnura verticalis Say, wih notes on certain parasites. *Entomologica am.* 17: 121-153.

JOHNSON, C., 1965. Mating and oviposition of damselflies in the laboratory. *Can. Ent.* 97: 321--326.

JOHNSON, C., 1966. Improvements for colonizing damselflies in the laboratory. *Tex. J. Sci.* 18: 179--183.

KOENIG, W.D., 1987. Lifetime reproductive success, selection, and the opportunity for selection in the white-tailed skimmer Plathemis lydia (Odonata: Libellulidae). *Evolution* 41: 22-36.

KRIEGER, V.F. & E. KRIEGER-LOIBL, 1958. Beiträge zum Verhalten von Ischnura elegans und Ischnura pumilio (Odonata). *Z. Tierpsychol.* 15: 82-93.

McVEY, M., 1988. The opportunity for sexual selection in a territorial dragonfly, Erythemis simplicicollis. *In*: T.H. Clutton-Brock, [Ed.], Reproductive success, pp. 44-58, cumulative references pp. 487-520, Univ. Chicago Press, Chicago.

MILLER, P.L., 1987a. Sperm competition in Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16: 201-207.

MILLER, P.L., 1987b. An examination of the prolonged copulations of Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16: 37-56.

MILLER, P.L., 1987c. *Dragonflies*. Cambridge Univ. Press, Cambridge.

MILLER, P.L. & C.A. MILLER, 1981. Field observations on copulatory behaviour in Zygoptera, with an examination of the structure and activity of the male genitalia. *Odonatologica* 10: 201-218.

OPPENHEIMER, S.D. & J.K. WAAGE, 1987. Hand-pairing: a new technique for obtaining copulations within and between Calopteryx species (Zygoptera: Calopterygidae). *Odonatologica* 16: 291--296.

PARR, M.J., 1973. Ecological studies of Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). 2. Survivorship, local movements and dispersal. *Odonatologica* 2: 159-174.

PARR, M.J. & M. PALMER, 1971. The sex ratios, mating frequencies and mating expectancies of three coenagriids (Odonata: Zygoptera) in Northern England. *Ent. scand.* 2: 191-204.

SOHAL, R.S., 1986. The rate of living theory: a contemporary interpretation. *In*: K.-G. Collatz & R.S. Sohal [Eds], Insect aging, Springer, Berlin [etc.].