CONTINUOUS AND STEPWISE OOCYTE PRODUCTION IN LIBELLULIDAE (ANISOPTERA)

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Compared to other insect groups, libellulids have a rather high mean number of ovarioles. In addition, the mean ovariole diameter differs greatly between and within species. In general, 2 different types of ovariole arrangement exist: (1) all developing oocytes mature and equal in size; in some species without, and in others with, surrounding connective tissue and (2) oocytes displaying gradual maturation, with only the outermost ovarioles mature. These differences have ecological consequences: the first arrangement occurs in spp. that have stepwise egg production. These spp. will lay one or more clutches, after which an interclutch interval of ovariole regrowth follows. Spp. with the second arrangement have continuous egg production and are able to lay at least some eggs all the time, reducing the length of interclutch intervals. However, no direct connection between mate-guarding strategies and ovariole arrangements can be seen. Nevertheless, it is believed that the process of ovariole maturation differs between these groups. It is concluded that ovary morphology in libellulids may exhibit evolutionary fixed traits, although the whole picture still remains complex. The ovariole arrangement may have a crucial impact on the reproductive ecology of the species.

INTRODUCTION

Clearly an important aspect of female reproductive success is egg production. Depending on evolutionary constraints or habitat requirements, eggs may vary in size, numbers and deposition rate, all of which factors have an impact on the survival rate of the offspring (WICKLUND et al., 1987; ALCOCK, 1993). In

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insects a larger egg size correlates negatively to female fecundity (ROFF, 2000; SANTO et al., 2001; SEKO & NAKASUJI, 2004) but results in larger larvae (TESSIER & CONSOLATTI, 1991; CORKUM & HANES, 1992; RICCI, 1995), and a faster growth rate of the larvae (SINERVO & McEDWARD, 1988; GIL-LOOLY & DODSON, 2000).

Insect ovaries consist of a group of cylindrical or tapering units, the ovarioles, which usually converge at the anterior end of the corresponding lateral oviduct (SNODGRASS, 1935). The anterior part of an ovariole is called the terminal filament; this anchors it to other ovarioles and to the body wall, dorsal diaphragm or, in some insects, the adipose tissue (SNODGRASS, 1935). The terminal filament is followed by the germarium, where cell division leading to the formation of oocytes takes place (TILLYARD, 1917; SNODGRASS, 1935). The main part of each ovariole is the vitellarium, where yolk proteins etc. are added to the growing oocyte (HAGEDORN & KUNKEL, 1979; BRENNAN et al., 1982; BÜNING, 1994). At the posterior end of the vitellarium, the oocytes are mature and the eggs ready to be deposited.

The number of ovarioles in an ovary varies between different insects. Usually the number is not high, four to eight ovarioles being typical (SNODGRASS, 1935). In some insect groups the number is, however, much higher. SZKLARZEWICZ et al. (2000) report between 50 and 92 ovarioles per ovary in the primitive aphid family Adelgidae and there are 85 in earwigs (TORNEUR, 1999), 100 to 200 in Hymenoptera and Diptera (SNODGRASS, 1935), 300 in hemipteran scale insects (SZKLARZEWICZ, 1998) and no less than 2400 have been reported from Isoptera (SNODGRASS, 1935). It thus seems as though systematic position has no influence on ovariole numbers.

It is known from studies on butterflies and *Drosophila* that the number of ovarioles may vary with female age (KARLSSON, 1987; WAYNE et al., 2005) as well as between different genetic strains of the species. The number of ovarioles is positively correlated with maximum daily fecundity (TELONIS-SCOTT et al., 2005; WAYNE et al., 2006). Hence, the ovariole number is a morphological character associated with fitness, at least in *D. melanogaster*. Current research on *D. melanogaster* has focused on examining the sources of variation in ovariole number in relation to fitness, with a view towards elucidating how segregating variation is maintained in natural populations (TELONIS-SCOTT et al., 2005). WAYNE et al., (2005) found significant genetic variation in ovariole numbers between and within collection sites for *D. melanogaster* and also a phenotypic plasticity in response to temperature. In the beetle *Dastarcus helophoroides* TOGASHI & ITABASHI (2005) found a strong correlation between ovariole number and female body length. WAYNE et al. (2006) found that the ovariole number increased in response to maternal starvation, suggesting an evolutionary association between maternal environment and the reproductive system of female progeny.

Dragonflies have panoistic ovaries, i.e., the simplest form of insect ovaries, where

all germinal cells develop into oocytes and nutritive cells are lacking (TILLYARD, 1917). Synthesis and release of mRNA, rRNA and energy reserves such as lipids, glycogen and the yolk protein vitellin is performed directly by the follicular epithelium (SNODGRASS, 1935; HAGEDORN & KUNKEL, 1979; BRENNAN et al., 1982; DETTNER & PETERS, 2003). The number of ovarioles in dragonflies is not known, and authors such as WINKELMANN (1973) simply state that they are 'numerous.' Dragonflies are known to have a continuous oocyte-production during their mature life span, and egg-laying periods are interspersed with periods of feeding away from their oviposition sites (e.g., THOMPSON, 1990).

In the family Libellulidae, two guarding types can be distinguished: non-contact guarding, in which the male accompanies the female, and tandem guarding, in which the male is physically coupled to the female during oviposition (CONRAD & PRITCHARD, 1992). In a previous paper involving one of us (Koch, formerly Schenk) it was shown that non-contact guarding species perform microhabitat selection by laying all their eggs within a small area (SCHENK et al., 2004). Tandem species by contrast, perform a spatial risk, laying their eggs in different areas (SCHENK et al., 2004). In addition, the mate-guarding types differ in egg size distribution within their egg clutches (SCHENK et al., 2004). This inspired us to investigate more links between morphological and behavioural traits associated with egg laying. Hence, in this paper we focused on three questions: First, how many ovarioles are present in dragonfly ovaries and how variable is this number within and between species? Second, we wanted to explore whether such basic morphological traits can be related to the type of mate-guarding. Finally, we were interested in the size variation of mature ovarioles, relating the number of ovarioles to known clutch size in different species.

MATERIAL AND METHODS

For this study sixteen species of libellulid dragonflies from three different countries (Germany, Japan and Namibia) were used, six non-contact guarding species and ten tandem guarding species (Tab. I). Where possible, we used several females in order to detect within species variation, if present, but for six of the selected species only single specimens were available. The species were chosen to represent eleven different genera distributed over several branches of the current tentative phylogenetic tree of the family (WARE et al., 2008; but cf., CARLE & KJER, 2002; and MISOF, 2002). Hence, we hoped to eliminate, or at least limit, any effects of phylogenetically fixed traits. Females were captured immediately after copulation and their head width measured to correct for size differences, as neither all species nor all specimens were of the same size. Most individuals were fixed in 70% alcohol, where they were subsequently stored, but some individuals were fixed in an alcohol-formaldehyde solution (80% : 4%) and, after 24 h, transferred to 80% alcohol for storage.

The abdomen was opened up lengthwise and put in a solution of 80% alcohol and 37% formaldehyde (4:1) for one hour before removing the ovaries. Thus, the ovaries were stabilised and could be dissected without breaking. This procedure was not necessary in the case of individuals previously fixed in alcohol-formaldehyde. Where possible, three pieces from the vitellarium of each individual (i.e., six sections; three from each ovarium) were dissected and taken through a normal dehydration protocol with 80 to 99.5% alcohol followed by xylene for paraffin embedding. We also transferred

some of the ovarioles to acetone after dehydration for embedding in TAAB 812 (epoxy) plastic.

For paraffin embedded ovaries we cut 8 μm microtome sections, which were stained with haematoxylin, while 4 μm microtome sections were cut from the plastic and stained with methylene blue. In both cases we used standard manual microtomes with steel knifes and standard staining protocols. Some samples deteriorated during the dehydration process and could not be used. Hence, one to six ovary sections of each female were used when counting ovarioles. The ovariole number is given per female, that is, the average of the sections used knowing that a female has two ovaries. The diameters of six to eleven mature ovarioles per female were measured. We defined mature ovarioles as being coated by epithelial cells (nurse cells). Ovarioles without well-defined nurse cells were thus considered immature (i.e. currently without growing oocytes). These were counted, but not measured. As the shape of a libellulid egg is oval, sometimes almost spherical (SAHLÉN, 1994; 1995), measuring all mature ovarioles in a section would result in varying diameters depending on what part of the developing egg was sectioned. However, as we were not concerned with actual egg size, we believe that the diameter will be equally variable for all studied species and, hence, that they are comparable. The measurements were performed using a Nikon Labophot 2 microscope and a Sony Exvawe-HAD-Digital camera in combination with the Easy Image™ Analysis program from Bergström Instruments AB, Lund, Sweden.

In order to analyse whether the number of ovarioles differs between the guarding types two ANCO-VAs were performed using mate-guarding type as the independent variable and number of ovarioles or ovariole diameter as dependent variables, using female head width as a covariable. In addition, we

Table I

Libellulid species in the study. Country of collection (C) given as G = Germany, J = Japan and N = Namibia. Number of individuals (n/n) used in measurements given as two figures: the first indicating number of individuals examined for mean number of ovarioles ± SD (number), the second indicating number of individuals examined for mean diameter of mature ovarioles ± SD (diam.). Species displaying non-contact guarding or tandem guarding behaviour are given as NC or TG respectively. Under Category 1a refers to all oocystes mature and no tissue between oocytes, 1b refers to all oocytes mature and connective tissue between oocytes, 2 refers to only peripheral oocytes mature. Note that both guarding types have members with ovaries of category 1 and 2

Species	C	Category	n/n	number	diam. [μm]	behaviour
Libellula depressa Linnaeus 1758	G	1b	6/2	57.6 ± 17.0	273.1 ± 34.7	NC
Orthetrum brachiale (Beauvois 1805)	N	1b	1/.1	76	177.2 ± 18.6	NC
O. julia Longfield 1955	N	1b	1/1	84	150.0 ± 21.3	NC
Leucorrhinia dubia (Vander Lind. 1825)	G	2	4/2	125.0 ± 19.4	78.9 ± 5.3	NC
Crocothemis erythraea (Brullé 1832)	N	2	2/2	136 ± 0	79.2 ± 18.9	NC
Trithemis kirbyi (Gerstaecker 1891)	N	2	3/3	171.3 ± 24.7	82.3 ± 19.1	NC
Sympetrum vulgatum (Linnaeus 1758)	G	1a	5/2	30.0 ± 6.9	169.6 ± 30.4	TG
S. frequens (Selys 1883)	J	1a	5/2	32.5 ± 9.6	176.6 ± 23.0	TG
S. infuscatum (Selys 1883)	J	1a	4/2	34.0 ± 7.5	266.4 ± 41.2	TG
S. danae (Sulzer 1776)	G	1a	1/1	36	172.6 ± 44.3	TG
Urothemis edwardsii (Selys 1849)	N	1b	1/1	38	158.4 ± 24.3	TG
Sympetrum fonscolombii (Selys 1840)	N	1a	4/2	46.0 ± 16.7	198.1 ± 43.2	TG
Diplacodes luminans (Karsch 1893)	N	1b	4/2	61.0 ± 13.7	243.5 ± 33.4	TG
Tramea basilaris (Beauvois 1805)	N	1b	1/1	68	214.5 ± 30.4	TG
Diplacodes lefebvrii (Rambur 1842)	N	2	1/1	146	154.73 ± 16.6	TG
Pantala flavescens (Fabricius 1798)	N	2	3/2	154 ± 87.0	153.7 ± 35.5	TG

calculated the mean number of mature oocytes per ovariole deposited in each egg clutch, using average clutch size values compiled from literature (SCHENK et al., 2004; KOCH & SUHLING, 2005; SCHENK & SÖNDGERATH, 2005; Tab. II). The mean number of ovarioles in these papers were derived from wild caught females in which clutch size theoretically may vary from zero to a very high value depending on time elapsed since the last egg laying event (cf., (SAHLÉN & SUHLING, 2002). However, in this paper we interpret the average values as representative for the species in question. Hence, we divided the observed average clutch size by the average number

Table II

Clutch size ($\overline{x}$ ± SD) of eight libellulid species from SCHENK et al. (2004), KOCH & SUHLING (2005) and SCHENK & SÖNDGERATH (2005). Number of clutches counted given as n

Species	clutch size	n
Leucorrhinia dubia	405.2 ± 170.7	17
S. fonscolombii	633.9 ± 382.1	41
Sympetrum danae	681.3 ± 275.6	10
Trithemis kirbyi	734.6 ± 488.3	50
Crocothemis erythraea	870.0 ± 363.5	6
S. vulgatum	930	1
Libellula depressa	984.3 ± 398.4	17
Pantala flavenscens	1246.0 ± 623.0	23

of mature ovarioles for each species. Using ANOVA we analysed whether the guarding types (independent variable) differed in the calculated mean number of mature oocytes per ovariole (dependent variable). All analyses in this paper were performed in SPSS 13.0.

RESULTS

In general, ovarioles in plastic sections were easier to count and measure than in paraffin embedded material. Although the plastic was regularly scratched during sectioning, which resulted in less sharp images, the tissue was better preserved and also withstood the cutting process better without breaking. There were no differences between the two fixation methods.

The mean number of ovarioles per female varied from 30 in *Sympetrum vulgatum* to 171.3 in *Trithemis kirbyi* (Tab. I). The specimen with the lowest number was a *S. frequens* with 22 ovarioles. The highest number was 254 in *Pantala flavescens*. In *P. flavescens* the standard diviation is over half the mean value, whereas in *T. kirbyi* (with a fairly similar mean) it is only about 15% of the mean (Tab. I). Average ovariole diameter varied from just 79 μm in *Leucorrhinia dubia* up to 273 μm in *Libellula depressa*. Both the intra- and interspecific size variation was, as expected, found to be high (Tab. I). Two different types of ovariole arrangement were found. In the first type the developing oocytes with their nurse cell epithelium were all mature and not varying much in size; all apparently ready to be deposited. No immature oocytes were observed. This could be divided into two sub-groups In the first no other tissue was present between the oocytes and this arrangement was found only in the *Sympetrum* species, which all had rather few ovarioles in their ovaries (30-46). In the second sub-group all the developing oocytes were surrounded by connective tissue (confirmed by staining with polychrome methylene blue and orcein). This kind of ovary was found in *Sympetrum luminans, L. depressa* (Fig. 1), *Orthetrum brachiale*, *O. julia*, *Tramea basilaris* and *Urothemis edwardsii*. In the second type of ovariole arrangement, the oocytes

varied a lot in size and appearance (Fig. 2). Most of the oocytes were immature, lacking nurse cell epithelium and visible yolk, and hence only a small proportion of them (ca 15-25%) contained egg cells ready for deposition. This type of ovary was found in *T. kirbyi*, *Crocothemis erythraea*, *L. dubia, Diplacodes lefebvrii* and *P. flavescens*. The mature ovarioles with oocytes ready to deposit were situated in two or three irregular layers on the perimeter of the ovaries with immature ovarioles on the inside. The smallest ovarioles were situated furthest from the body wall, near the centre of the body, i.e., next to the alimentary canal (Fig. 2).

The mean number of ovarioles differed significantly between the two mate-guarding types ($F_{1,39}$ = 11.41, P < 0.002). In non-contact guarding species the number of ovarioles was higher than in tandem species (Tab. I). Additionally, the ovariole diameter differed significantly between the two mate-guarding types ($F_{1,139}$ = 40.23, P < 0.0005). In non-contact guarding species the mean ovariole diameter was smaller, but varied more than in tandem species (Tab. I). Species size had no influence on the mean number of ovarioles ($F_{1,39}$ = 2.43, P = 0.127) but it affected the mean ovariole diameter ($F_{1,139}$ = 49.12, P < 0.0005). The correlation between species size and ovariole number is not perfect but, on the whole, larger species had larger ovarioles. The calculated mean number of mature oocytes per ovariole deposited in each egg clutch of non-contact guarding species was 29.0 for *C. erythraea*, 13.5 for *L. dubia*, 17.1 for *L. depressa*, and 26.2 for *T. kirbyi*. In tandem guarding species the numbers were 31.1 for *P. flavescens*, 17.8 for *Sympetrum danae*, 21.1 for *S. fonscolombii* and 31.0 for *S. vulgatum*. The number of mature oocytes per ovariole did not differ between the two guarding types ($F_{1,6}$ = 0.56, P = 0.477).
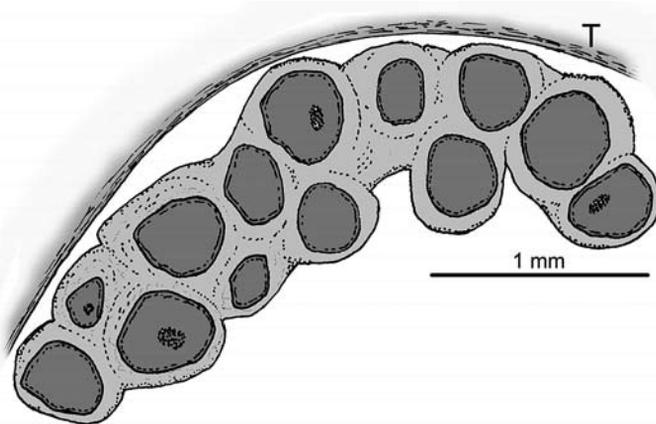

Fig. 1 Cross section through one ovary of *Libellula depressa* at the level of abdominal segment 7, re-drawn from a paraffin mount. In this specimen there are 13 in the ovary (dark grey), all mature and surrounded by a thick layer of connective tissue (light grey). All oocytes are of almost the same size and at the same stage of maturation. T. Approximate position of the abdominal tergite.

Looking at the two types of reproductive behaviour in libellulids, tandem guarding and non-contact guarding, we see that there is no clear division between ovariole type and guarding types. Both guarding types have members with ovaries of both major groups (Tab. I). Considering our measurements (Tab. I) we see that there are some groups of species which seem to share the morphological features of their ovaries. One obvious group consists of *L. depressa* and the *Orthetrum* species, which have fewer ovarioles than the other non-contact guarding species. Their ovariole morphology is also the same as in most other tandem species. In contrast, *P. flavescens* and *Diplacodes lefebvrii* have a higher ovariole number than the other tandem species. In the former species even the ovariole appearance is that of most non-contact species.

DISCUSSION

In this study, libellulid dragonflies were found to have between 22 and 254 ovarioles per female. In this respect dragonflies differ from many other insects, in which the numbers are much lower (SNODGRASS, 1935). As in *Drosophila melanogaster* (e.g., CARLSON & HARSHMAN, 1999; WAYNE et al., 2005) the ovariole number varied within and between species, probably depending on female age and genetic differences as well as phenotypic responses as suggested in
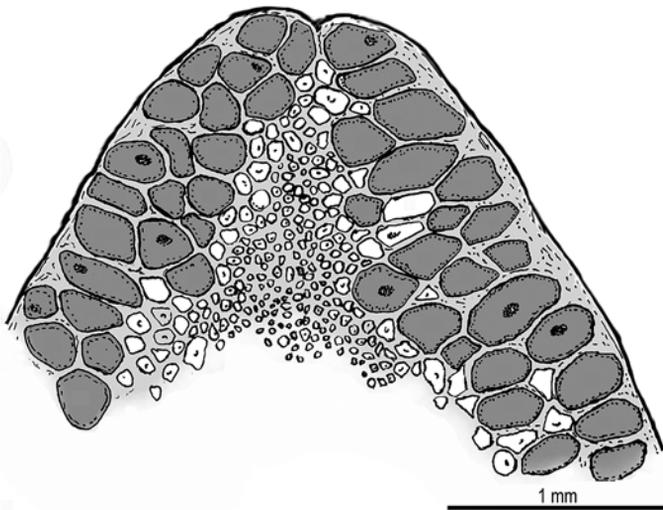
Fig. 2 Cross section through a pair of ovaries of *Pantala flavescens,* at the level of abdominal segment 7, redrawn from a paraffin mount. In this specimen there are 23 mature oocytes (dark grey) in each ovary arranged in double or triple rows in the periphery, next to the body wall. In addition there are 208 immature oocytes (white), the smallest toward the centre of the drawing, which is close to the alimentary canal. It is clear that oocytes mature continuously outwards from the inner region of the ovaries. The light grey areas between the oocytes consist of connective tissue.

studies on other insects. Species size had no influence on the number of ovarioles. Large species such as *Libellula depressa* and *Orthetrum brachiale* had few ovarioles; smaller species such as *Trithemis kirbyi* had a high number of ovarioles and the smallest species, *Sympetrum danae*, had only 36 ovarioles, which is among the lowest numbers found in the study. In general, larger insects have more ovarioles than small ones (CHAPMAN, 1988; RICHARDS, 1994) and the number is often reported as rather constant within a species. Hence, these general rules do not seem to apply to libellulid dragonflies. Instead, ovariole number and arrangement in libellulids probably correlate to phylogeny (cf., below).

The mean ovariole diameters noted in this study were often smaller than the diameters of deposited eggs from the same or closely related species found in other papers (e.g., SAHLÉN, 1994; SAHLÉN & SUHLING, 2002). We explain these size differences by the fact that we did not always section through the widest part of the eggs and, hence, the values should not be seen as corresponding to the actual egg diameters.

Looking at ovariole orientation and development within the ovaries, we found two different arrangements: The first type, with all developing oocytes mature and equal in size (Fig. 2) encompass species which seem to be adapted to deposit a large number of eggs during a short time span. As no immature ovarioles were present, we assume that these species should lay one or more egg clutches, after which a period (interclutch interval) of ovariole regrowth should follow. The old epithelial cells and, hence, most probably also any adjoining connective, are degraded before egg deposition (BÜNING, 1994). In the second group there is a gradual maturation process of the ovarioles with the outermost ovarioles (ca 15-25% of them) mature with less mature ovarioles inside them, the youngest, most immature ovarioles being placed at the centre of the female body close to the rectum (Fig. 2). We think that this arrangement makes it possible for these species to have a more continuous egg production, that is, the ovarioles mature in succession, which enables the females to lay a few eggs at all times, reducing the length of the interclutch intervals. So far all odonates have been assumed to have a continuous oocyte production during their whole mature life span, though the egg production speed as well as the size of the eggs of investigated species decline with increasing female age (e.g., WATANABE & ADACHI, 1987; WATANABE & HIGASHI, 1989; THOMPSON, 1990; HIGASHI & WATANABE, 1993; ELZINGA et al., 2005). However, we propose a division into species which we define as having stepwise egg production (those with numerous immature ovarioles and only a few mature oocytes) and those with continuous egg production (with all of the oocytes mature).

Both guarding types have members with ovaries of both major groups. There are some groups of species which seem to share the morphological features of their ovaries. One obvious group consists of *L. depressa* and the *Orthetrum* species, which have fewer ovarioles than the other non-contact guarding species and

an ovariole appearance as in most other tandem species. In contrast, *P. flavescens* and *D. lefebvrii* have a higher ovariole number than the other tandem species and an ovariole appearance is that of most non-contact species. Hence we conclude that ovary morphology in this family exhibits evolutionary fixed traits.

The two reproductive strategies do, however, differ in the morphology of the ovaries in other ways. Looking at clutch size, it is known that the number of eggs deposited does not differ between the mate guarding types (KOCH & SUHLING, 2005; SCHENK & SÖNDGERATH, 2005) but tandem species produce larger oocytes / eggs (KOCH & SUHLING 2005). To produce the same number of slightly larger eggs requires higher costs for egg production. As there is no clear distinction in ovariole numbers between the reproductive groups and nothing is known of metabolism speed variation in dragonflies, the only way for the animals to compensate the production of larger eggs would be longer breaks between oviposition events. Even so, the number of eggs deposited at each event is high in both groups, our calculated values of between 13 and 31 mature oocytes per ovary probably not showing the whole variation within the family. These values give an indication of how much energy is needed for egg production by the females when on a foraging / resting period between egg laying events, a period normally lasting one or two days (e.g., SIVA-JOTHY et al., 1998).

Several authors have described the morphology of the odonate ovaries (e.g., PRASAD & SRIVASTAVA, 1961; ANDO, 1962; TILLYARD, 1917; BÜNING, 1994) but these descriptive papers have not provided any way of explaining the fact that the egg size distribution differs between the two mate-guarding types. SCHENK et al., (2004) showed that, in clutches of species which perform non-contact guarding, the egg size is inversely proportional to the order of laying, whereas it is randomly distributed in tandem species. The idea of focusing on stepwise versus continuous egg production in these groups might be a thought-provoking idea, and as we only looked at a limited number of species we may not see the whole picture yet.

The differences in oviposition behaviour (SCHENK et al., 2004), life history traits (SCHENK & SÖNDGERATH, 2005) and morphological traits (KOCH & SUHLING, 2005) between the two guarding types examined may also, together with the morphological differences observed in this study, be related to migration. The non-contact guarding species in this study belongs, with the exception of *L. depressa*, to the residents or facultative migrants (CORBET, 1999; JOHANSSON & SUHLING, 2004). Interestingly *L. depressa* had the lowest number of ovarioles and the highest ovariole diameter of all the studied non-contact guarding species and would hence fit better among the tandem guarding species. Also phylogeny shows that, within the non-contact guarding species, *L. depressa* and the two *Orthetrum species* seem to establish a group of their own (CARLE & KJER, 2002; MISOF, 2002; Fig. 1). In contrast, the tandem guarding species studied are obligate migrants (CORBET, 1999; JOHANSSON & SUHLING, 2004).

Migrating individuals often have a lower fecundity than residents as a result of large wings, flight muscles and the cost of flying (DENNO et al., 1991; ROFF & FAIRBAIRN, 1991; ROFF, 1994; ROFF & BRADFORD, 1996), and may also suffer from a higher mortality while in transit (HOPPER, 1999). If we assume we are studying the effects of migration we note that, in addition to the long distance flights to reach the reproduction sites, tandem / obligate migrant species seem to invest more energy in egg production due to producing larger oocytes / eggs while maintaining the same clutch size. It might therefore be worthwhile to compare the total egg production / fitness during the whole life span between the two reproductive strategies. As the two strategies have coexisted for a long time, we must assume that the tandem system is beneficial under certain circumstances. Most studies have discussed mating systems with the main focus on sexual selection (WAAGE, 1984; FINCKE et al., 1997; DAY, 2000; GAVRILETS et al., 2001; PANHUIS et al., 2001; TURELLI et al., 2001; HÄRDLING & KAITALA, 2004). To focus more on natural selection and morphology might bring new insights into the discussion of mating systems. Natural selection is a fundamental source of evolutionary diversification and a mechanism of speciation (SCHLUTER, 2000; TURELLI et al., 2001; BLACKLEDGE & GILLESPIE, 2004). The benefit of tandem guarding might be a greater ability to change microhabitat during oviposition (BUSKIRK & SHERMAN, 1984), which allows spatial risk spreading and therefore a better adaptation to variable and temporary habitats (WELLBORN & ROBINSON, 1987; STEARNS, 1992; LAAKSONEN et al., 2002; EINUM & FLEMING, 2004; LAAKSONEN, 2004). Recapitulating, this study gives more evidence of the complex interactions between behavioural, life history and morphological traits, and migration pattern. Hence, when conducting such studies it is important not to focus on single, isolated traits, as the performance of individuals may be regulated by multiple traits.

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