The male dimorphism in the dwarf spider *Oedothorax gibbosus* (Blackwall, 1841) (Erigoninae, Linyphiidae, Araneae): Results of laboratory rearing experiments

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ABSTRACT. *Oedothorax gibbosus* is a dwarf spider bound to oligo- and mesotrophic alder carrs. Two male morphs occur: *gibbosus*, characterised by a hunch and a hairy groove on its carapace, and *tuberosus* without these features. The hairy groove is supposedly of importance during gustatorial courtship behaviour.

Earlier studies indicated that this dimorphism was presumably determined by a di-allelic gene only expressed in the male sex. Allele G for *gibbosus* is dominant over allele g for *tuberosus*. An elaborate laboratory rearing experiment was set up to test this hypothesis, which was based on only a small number of observations. Analysis of a large number of family trees was in agreement with this model of inheritance.

A small fraction of the reared spiders (5%) needed a fifth moult to reach the adult instar. This is the first time that an exception to the normal number of four moults in dwarf spiders of the subfamily Erigoninae has been observed. The dwarf spiders that moulted five times also hatched significantly earlier, and this probably made an additional moult necessary.

The juvenile phase of the *gibbosus* males was significantly longer than that of the *tuberosus* males. The juvenile phase is the period between the emergence of the spiders and the last moult.

Two possible mechanisms compensating for the advantage of *gibbosus* in sexual selection were observed: sex ratio distortion in favour of the female sex in *tuberosus*-genotypes, and shorter juvenile development.

KEY WORDS: Araneae, Erigoninae, male dimorphism, sexual selection, one-locus system, fifth moult, juvenile development, sex ratio distortion, Q10 rule.

INTRODUCTION

Oedothorax gibbosus is a rare dwarf spider species in Flanders, which occurs in wet to very wet habitats (DE KEER & MAELFAIT, 1989; ALDERWEIRELDT, 1992) such as oligo- and mesotrophic alder carrs. They occur between litter and mosses in the immediate vicinity of open water. The males are smaller than the females. The abdomen of *Oedothorax gibbosus* is black and gleaming, and while walking the dwarf spider sometimes raises the abdomen. When the dwarf spider reaches adulthood it is approximately 3 mm in length.

Oedothorax gibbosus is characterised by male dimorphism. The *gibbosus* morph has a protuberance on the last third of the carapace, anterior to which is a deep notch surrounded and filled by long black silky hairs. This hairy groove probably secretes a fluid that is important for the gustatoric courtship behaviour, and *gibbosus* would therefore have a reproductive advantage (HEINEMANN & UHL, 2000). The *tuberosus* morph does not have these features and its carapace is more or less convex. Previously, both morphs were considered to be two different species, *Oedothorax tuberosus* (Blackwall, 1841) and *Oedothorax gibbosus* (Blackwall, 1841), which could only be distinguished on the basis of the morphology of the males. DE KEER & MAELFAIT (1988) proved the male dimor-

phism in *Oedothorax gibbosus*: both morphs hatched from one cocoon collected in the field.

Polymorphism according to FORD (1945) is the coexistence of two or more discontinuous genetically-determined morphs. Dimorphism within one sex is rare, and is generally related to genetically-based alternative mating tactics (ANDERSSON, 1994). The presence of two morphs in one population can only be stable if both morphs have the same average fitness (GADGIL, 1972). HEINEMANN & UHL (2000) proved on the basis of carapace measures that *tuberosus* and *gibbosus* are two discontinuous morphs and that the so-called intermediate morphs according to ROBERTS (1987) do not exist in *Oedothorax gibbosus.*

MAELFAIT et al. (1990) proposed, based on only a small number of observations, that the male dimorphism in *Oedothorax gibbosus* is determined by a di-allelic gene only expressed in the male sex. In this model allele G for *gibbosus* is dominant over allele g for *tuberosus* (MAELFAIT et al., 1990). Here, we report data of an elaborate laboratory rearing to test this hypothesis. We also present some other aspects of the development of *Oedothorax gibbosus*.

MATERIAL AND METHODS

The dwarf spiders were caught in the public nature reserve "Het Walenbos" at Tielt-Winge, 30 km north-east of Brussels. Situated on the right bank of the river "de Motte", it is one of the most important river-associated woods of Flanders. The presence of oligo- and mesotrophic alder carrs is typical for "Het Walenbos". The dwarf spiders were caught by hand in such alder carr on October 29, 1998 and on April 9, 1999. The dwarf spiders were placed individually in petri-dishes (3.5 cm diameter and 1 cm height) with a thin bottom of carbonic plaster to allow an observer to see whether the bottom is still humid. This is necessary because of the low resistance of *Oedothorax gibbosus* to desiccation. The petri-dishes were moistened regularly to maintain a relative humidity near 100%. The spiders were kept in a climatic chamber at a temperature of circa 18°C and a photoperiod L:D of 16:8. We opted for 18°C because this resembles the mean wood temperature in summer. Before the second moult spiders were fed at least every two days with four springtails (*Isotoma viridis* among others); after the second moult they were given three fruit flies per day. We reared three generations of descendants starting with 15 males and 15 females from the field. At the start of the first, second and third generation, there were respectively 245, 461 and 223 spiders. To cross one male and one female we placed them in a large petri-dish (5,5 cm diameter and 1.2 cm height). The development of the dwarf spiders, such as the occurrence of the moults and the production of cocoons, was registered accurately using a WILD-binocular dissecting microscope and a cold light source. The development of the dwarf spiders is subdivided into the cocoon phase and the (free) juvenile phase.

The cocoon phase is the period between the production of the cocoon and the emergence of the spiders; the (free) juvenile phase is the period between the emergence of the spiders and the last moult.

The following statistical tests were used: t-test with independent variables, Mann-Whitney-U-test, Kolmogorov-Smirnov test, χ^2-test and two-way-ANOVA. We also used the formula of WONNACOTT & WONNACOTT (1990) to calculate the 95% confidence intervals for a proportion:

95% confidence interval = $p \pm 1,96 \sqrt{\dfrac{p(1-p)}{n}}$
(p = proportion, n = sample size)

RESULTS

Mendelian inheritance of male dimorphism

The monogenic di-allelic inheritance model of the male dimorphism, as proposed by MAELFAIT et al. (1990), yields six possible crossing types (Table 1). Especially crossing type 3 shows that the allele G for *gibbosus* is dominant and that the allele g for *tuberosus* is recessive, because this is the only crossing type between a *gibbosus* and a *tuberosus* that results in descendants that all have the *gibbosus* morph. Using the data of the laboratory rearing we have put several family trees together, to investigate if the morph division of the descendants agrees with the inheritance model. To determine the genotype of each female and male we assume that there is no differential mortality in the species *Oedothorax gibbosus* and that a female emerging from a cocoon in which all males have

TABLE 1

The different crossing types with corresponding phenotypes *gibbosus* (*gib*) and *tuberosus* (*tub*) (MAELFAIT et al., 1990)

juveniles / *gibbosus* males / *tuberosus* males / females

| male *tuberosus* gg | female *tuberosus* gg | male *gibbosus* GG of Gg | female *gibbosus* Gg of GG | male *tuberosus* gg | female *tuberosus* gg | male *tuberosus* gg | female *gibbosus* Gg |

male *tuberosus* gg — 0 / 0 / 7 / 0

female *gibbosus* Gg — 16 / 3 / 0 / 7 + 2 males ?

male *tuberosus* gg — 4 / 0 / 10 / 16

female *tuberosus* gg — 6 / 1 / 0 / 13 + 1 male ?

male *gibbosus* Gg — 3 / 10/ 12 / 15

female *tuberosus* gg — 4 / 0 / 3 / 13
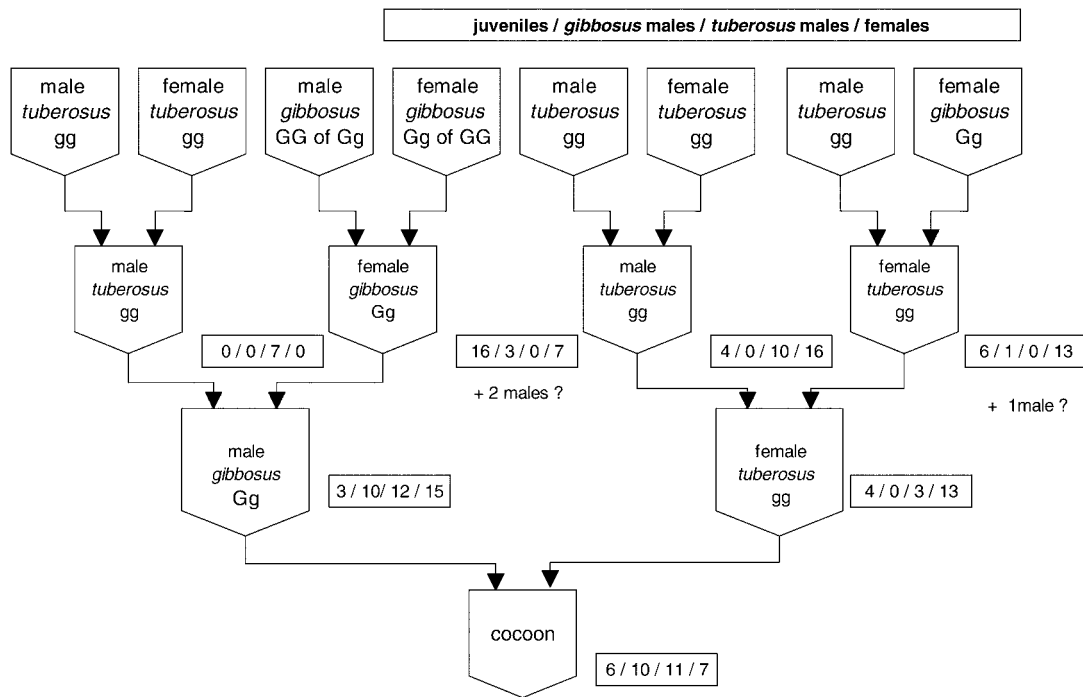
cocoon — 6 / 10 / 11 / 7

Fig . 1. – An example of a family tree. In this family tree the sex, the code, the morph and the genotype of each spider are indicated. The numbers in the rectangles represent respectively the number of juveniles (= spiders which died before adulthood), the number of *gibbosus* males, the number of *tuberosus* males and the number of females in each cocoon.

the *gibbosus* morph, also has a *gibbosus* genotype; the same applies to the *tuberosus* morph. We obtained ten family trees from the progenitors till the third generation and two family trees till the second generation. Fig. 1 shows an example of such a family tree. Five of the six crossing types appear in these family trees. The first, although self-evident, crossing type (GG x GG $\rightarrow$ GG, GG, GG, GG) did not appear in any family tree. Especially crossing types 4, 5 and 6 are in majority. All family trees were in agreement with the postulated di-allelic monogenic inheritance of male dimorphism by MAELFAIT et al. (1990).

The fifth moult

Approximately 5% of the dwarf spiders moulted five times in the laboratory. The spiders that moulted five times needed this last moult effectively to reach adulthood. The frequency of moulting five times was approximately equal in males and females; 11 of 165 males and 20 of 291 females moulted five times. According to the χ^2-test these proportions are not significantly different (df = 1, p = 0,937). Significantly more *tuberosus* males than *gibbosus* males moulted five times (χ^2-test: df = 1, p = 0,014); ten of 87 *tuberosus* males and one of 78 *gibbosus* males moulted five times. The number of moults has an important effect on the cocoon phase. This could not be investigated in the first generation of dwarf spiders because in this generation there was no single case of a fifth moult. In the third generation the cocoon phase was significantly shorter in the spiders that moulted five times than in the spiders that moulted four times (t-test: $n_{5moults}$ = 12, $n_{4moults}$ = 73, df = 83, p = 0,002; U-test: $n_{5moults}$ = 12, $n_{4moults}$ = 73, U = 223, Z = -3,05, p = 0,002). In the spiders with five moults the mean cocoon phase was 16 ± 1 days; on the other hand this was 18 ± 2 days in the spiders with four moults. There was also a significant effect of the number of moults on the cocoon phase according to the t-test in the second generation (t-test: $n_{5moults}$ = 18, $n_{4moults}$ = 260, df = 276, p = 0,032), although this effect is not significant according to the U-test (U-test: $n_{5moults}$ = 18, $n_{4moults}$ = 260, u = 1551, Z = -0,64, p = 0,523).

The juvenile development

The ANOVA test analysis of the effect of the (male) spider morph and the generation on the juvenile phase, yielded the following results. The juvenile phase of *gibbosus* was, in generations 1 and 2 (df Effect = 1, df Error = 351), significantly longer than the juvenile phase of *tuberosus* (F= 11,106; p= 0,001). The same result was obtained for generations 2 and 3 (df Effect = 1, df Error = 103) and for generations 1 and 3 (df Effect = 1, df Error = 48). The t-test as well as the U-test confirms this result. Table 2 shows the mean juvenile phase of each generation. The ANOVA test that analyses the effect of the crossing type (crossing type 1, 4 and 6) and the sex on the juvenile phase in generation 2, shows that the crossing type, as was to be expected, had a significant effect on the juvenile phase (df Effect = 2, df Error = 205, F = 8,8, p = 0,0002). Only the three most com-

monly occurring crossing types were used for this test, and the spiders coming from crossing types that yielded more *gibbosus* offspring had a significant longer juvenile phase (fig. 2). This test also shows that females had a significant longer juvenile phase than did males (df Effect = 1, df Error = 205, F = 12,0, p = 0,0006) and that there was no significant interaction between the effect of crossing type and sex on the juvenile phase (df Effect = 2, df Error = 205, F = 1, 9, p = 0,16). This last observation is very important because it means that the effect on the juvenile phase also holds for the females although the genotype of dimorphism is not expressed in their phenotype. So-called '*gibbosus* females', this means females that have a genotype with at least one G, have also a significantly longer juvenile phase.

Sex ratio distortion in favour of the female sex

In both field catches of the spiders, the female sex predominated; in the first catch the percentage of females was 66%, in the second catch 61%. So the sex ratio in the population of "Het Walenbos" is approximately 1 to 2; this means theoretically that for each male there are two females available. This sex ratio was also maintained in the dwarf spiders of the three laboratory generations: in generations 1, 2 and 3 the sex ratios were respectively 47 to 98, 107 to 196 and 58 to 95. According to the χ^2-test the sex ratios of the three different generations are not significantly different (df = 2, p = 0,614). Table 3 shows the sex ratio of the two most commonly occurring crossing types in the laboratory rearing. In crossing type 5 a distinction is made between the situation that Gg or gg is the paternal or the maternal genotype. If gg is the maternal genotype, the sex ratio is approximately fifty-fifty. On the other hand the sex ratio is shifted in favour of the female sex if gg is the paternal genotype. In crossing type 6 gg is the paternal as well

TABLE 2

Survey of the mean values ± standard deviation of cocoon phase and juvenile phase in each generation separately and in all generations together

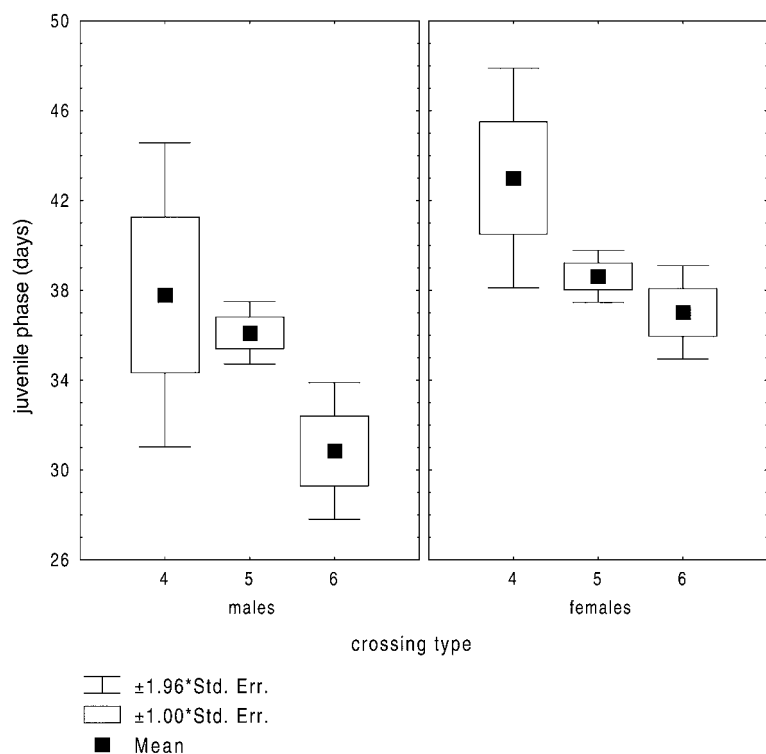| generation	cocoon phase	juvenile phase
1	18 ± 2	42 ± 4
2	15 ± 4	50 ± 6
3	18 ± 2	50 ± 8
1+2+3	17 ± 3	44 ± 6

±1.96*Std. Err.
±1.00*Std. Err.
■ Mean

Fig. 2. – The effect of crossing type and sex on the juvenile phase in generation 2. Each framework represents a sex, each box-and-whicker a crossing type. The juvenile phase is significantly longer in the spiders of crossing type 4 and 5 (*gib*) than those of crossing type 6 (*tub*). The juvenile phase of the spiders of crossing type 4 (2x G) is almost significant longer than those of crossing type 4 (1x G).

TABLE 3

Sex ratio of the two most frequent crossing types in the laboratory rearing. In crossing type 5 a distinction is made between the situation that Gg or gg is the paternal or maternal genotype *The sex ratio is here expressed as ratio of total number of male offspring divided by the total number of offspring for each crossing type (families = number of observed families for each crossing type, males = sum of males of all families for each crossing type, females = sum of females of all families for each crossing type)

crossing type	families	male genotype	female genotype	males	females	sex ratio*
5	8	Gg	gg	48	53	48 %
5	12	gg	Gg	65	115	36 %
6	18	gg	gg	52	110	32 %

as the maternal genotype. The sex ratio in this crossing type is completely shifted in favour of the female sex (Table 3). According to the χ^2-test there is a significant difference between those three sex ratios (df = 2, p = 0,039). The confidence intervals of the sex ratio for every situation overlap partly, but if the paternal genotype is gg and in the case of crossing type gg x gg, the confidence interval is situated under the fifty-fifty sex division (Fig. 3).
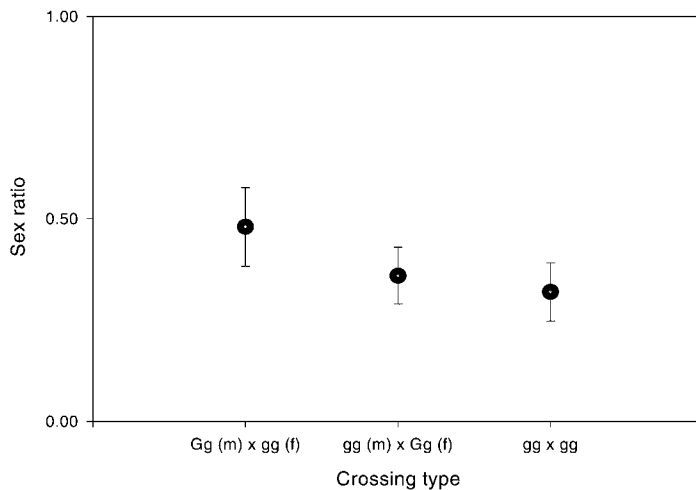

Fig. 3. – The confidence intervals of the sex ratios of the two most occurring crossing types in the laboratory rearing. Dots indicate the ratio of total number of male offspring divided by the total number of offspring for each crossing type. Error bars indicate 95% confidence intervals calculated with the formula of WONNACOTT & WONNACOTT (1990).

DISCUSSION

The monogenic, di-allelic inheritance model of the male dimorphism in the dwarf spider *Oedothorax gibbosus* is confirmed by the analysis of the different family trees. Five of the six possible crossing types appeared in the laboratory rearing. The fact that the very evident crossing type 1 (GG x GG ➤ GG, GG, GG, GG) is absent in the rearing, is probably due to chance. The fact that the longer juvenile phase also appears in females with a *gibbosus* father, points out that the genotype of dimorphism also influences the female sex, although this genotype is not expressed phenotypically in females. A great disadvantage during the research was high mortality in the dwarf spiders. This may have caused a small inaccuracy in the results.

The representatives of the Linyphiidae are characterised by a great constancy of four moults, although there have been exceptions found: *Stemonyphantes lineatus* moults nearly always five times and *Floronia bucculenta* mostly moults also five times (SCHAEFER, 1987). Both species belong to the subfamily of the Linyphiinae, which are characterised by larger sized spiders. *Oedothorax gibbosus,* however, is a representative of the subfamily Erigoninae which contains smaller dwarf spiders. It is surprising that such a little dwarf spider as *Oedothorax gib-*

bosus sometimes needs an additional moult because according to ROBERTS (1995) smaller spiders moult fewer times than do larger spiders, but SCHAEFER (1987) already points out that there is no clear correlation between the size of the spider and the number of moults. In *Oedothorax gibbosus* the fifth moult occurs only occasionally, but this fifth moult is necessary to reach adulthood and maturity. The dwarf spiders that moult five times also hatch significantly earlier, and this probably makes an additional moult necessary. This is the first time that an exception to the normal number of four moults in dwarf spiders of the subfamily Erigoninae has been observed.

The juvenile phase of *gibbosus* males is significantly longer than that of *tuberosus* males. This is perhaps necessary for the production of the hunch and the hairy groove, which may need a high energy investment. The shorter development rate is a possible advantage for *tuberosus*. When temperature is high the development rate increases and the development time decreases. One can demonstrate on the basis of the Q10 rule that the advantage of *tuberosus* males would be greater if it is colder. Q10 is the factor by which a physiological function increases as response to a temperature rise of 10 °C (PROSSER, 1973). The factor Q10 mostly varies from 2 to 3 (PULZ, 1987). In other words if the temperature increases 10 °C the development rate, for example, increases by a factor of 2 to 3. Let's suppose that the factor Q10 for the development in *Oedothorax gibbosus* is 3. When it is 18 °C the mean juvenile phase of *gibbosus* males and *tuberosus* males is respectively 44 and 37 days. This is a difference in juvenile development time of 7 days. If the temperature would increase 5 °C (23 °C), the development rate would increase by a factor of 1.5 and the development time would be decreased by a factor of 1.5. The difference in juvenile development time between both morphs would then only be 4 days. There is thus a large overlap between the appearance of mature *tuberosus* and *gibbosus* males. Because of the presence of the hairy groove and the dominance of *gibbosus,* a *gibbosus* male has an advantage from the moment that it can copulate; therefore the *tuberosus* male would have little advantage with a lead of 4 days. If the temperature would decrease 5 °C (13 °C) the development rate would decrease by a factor of 1.5 and the development time would increase by a factor of 1.5. The difference in juvenile development time would be 10 days. *Tuberosus* males would have a greater lead and could fertilise the mature females earlier. This scenario is purely hypothetical and has to be further investigated.

Normally one would expect that there are as many males as females in a population, because according to FISHER (1930) natural selection promotes a fifty-fifty sex ratio. In "Het Walenbos" the sex ratio is 1 to 2 and one can deduce from the results that the *tuberosus* morph probably

can compensate the genetic dominance of the *gibbosus* morph by a sex ratio distortion in favour of the female sex. The mechanism of this is still unknown, but there are many known examples from the literature in which parents influence the sex ratio of their offspring (TRIVERS & WILLARD, 1973; CLARKE, 1978; CLUTTON-BROCK et al.*,* 1984; SUGIARA, 1994; SVENSSON & NILSSON, 1996; CAZEMAJOR et al., 1997; KOMDEUR et al., 1997; BRADBURY & BLAKEY, 1998; KILNER, 1998; WERREN & BEUKEBOOM, 1998).

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