Case of Unilateral Wing Formation in the Female of the Glowworm *Lampyris noctiluca*

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ABSTRACT   On July 27, 1999, the first author found a unilaterally winged adult glowworm in a park in the city of Mainz. Except for the wings on the left side, the specimen exhibited female characteristics that extended to external sexual appendages, the lantern and the gonads. The internal organization showed some remarkable differences between right (wingless) and left (winged) side. The right ovary contained three times more mature eggs than the left side and the volume of the corpus allatum of this side was about one-third larger than that of the left side. This suggests that aptery and egg maturation are affected by corpus allatum activity, i.e., juvenile hormone production. The findings do not support the hypothesis of Naisse ([1966] Arch Biol Liège 77:139–201) that wing formation, as a secondary male characteristic, is controlled by an androgenic hormone from the testes in the glowworm. Thus, the observations on this exceptional specimen have implications for the current hypotheses concerning the control of sexual wing dimorphism in *Lampyris noctiluca*. J. Morphol. 257:254–258, 2003.   © 2003 Wiley-Liss, Inc.

KEY WORDS: sexual wing dimorphism; sexual differentiation; endocrine glands; corpora allata; *Lampyris noctiluca*.

Glowworms and fireflies are most well known for using light signals to attract mating partners or for other bizarre behavior: firefly "femmes fatales," for instance, mimic the light signal of a related genus to entrap those males and prey on them (Eisner et al., 1997). But lampyrid beetles have still other exceptional features. Most of them, and in particular *Lampyris noctiluca*, have a strong sexual dimorphism that becomes apparent at the pupal stage and is most prominent in adults. Whereas the male of *L. noctiluca* has well-developed wings and is capable of flight, the female, which is much larger than the male, lacks wings completely. The sexes differ greatly in their sexual appendages and, although slightly, in the appearance of the lantern. Thus, the species can serve as a model for studying both the regulation of the differentiation of primary and secondary sexual characteristics as well as the suppression of wing formation in the female. In fact, both aspects, which may be interrelated, have been studied in *L. noctiluca*. Davydova (1967) examined the role of juvenile hormone (JH) in wing formation during metamorphosis, and in a series of investigations Naisse (1966a–c, 1969) analyzed the endocrine control of the differentiation of primary and secondary sexual characteristics. According to Naisse (1966a), *L. noctiluca* represents a unique case in the direction of sexual differentiation among insects. Up to the third larval instar (L3) the structure of gonads and external morphological features cannot be distinguished between the sexes. In early L4, male gonads develop an apical tissue that synthesizes and releases an androgenic hormone into the hemolymph. This still uncharacterized hormone supposedly induces the male gonads to become functional testes, and somatic tissue to form the external genitalia and secondary sexual characteristics, including wings. If immature female gonads are implanted into male larvae with active apical tissue, the implanted female gonads will develop into testes. If testes with active apical tissue are implanted into female larvae, the female hosts will transform into complete male adults. The specimen introduced here bears wings (although crippled) on the left side, whereas they are totally absent on the right side. If the finding of Naisse (1966a) is correct, i.e., that wing formation is also controlled by the apical testis tissue, the wing-bearing side should contain a testis, at least a rudimentary one. Thus, the described specimen presents a challenge to the long-standing statements of Naisse. Recent studies on the testis differentiation in the glowworm cast serious doubts on the proclaimed source of the androgenic hormone (Balles et al., 2002).

MATERIALS AND METHODS

We have raised *Lampyris noctiluca* in our laboratory continuously for several years (for details, see Maas et al., 2001). Although we have seen thousands of specimens, we never observed a glowworm with the abnormalities described here in a beetle collected in its natural habitat. The first author detected this beetle in the course of observational studies in the summer (July

27) of 1999 in a park within the city of Mainz. The beetle was brought into the laboratory and observed for another day. It showed the normal activities: hiding under bark and vivid locomotion when removed from the hiding place. Adult *L. noctiluca* no longer feed and are short-lived. They mate soon after eclosion (if mating partners meet), readily deposit eggs, and die within about 10 days. This unilaterally winged specimen was kept together with adult males and females and also larvae for 1 day. Mating of the specimen was not observed during this period, although it exhibited strong light emission. Since we planned a histological examination of the specimen, we did not want to take the risk of its sudden death. Therefore, we dissected it and fixed its tissues 1 day after collection.

For examination of the internal organization, the tergum was removed from the abdomen and thorax. The body cavity was flooded with Grace's Insect Medium (Sigma Chemie, Deisenhofen, Germany) and the internal organs photographed. Then several organs were dissected and transferred into aqueous Bouin's fluid. Brain, retrocerebral complex, subesophageal ganglion, foregut, and the anterior part of the midgut were removed in toto and prepared for serial sections. The organ complex was embedded in methacrylate. Serial sections of 4 μm thickness were stained with PAF (paraldehyde-fuchsin) and light green SF (Sigma Chemie) for neurosecretions. The whole procedure—fixation, embedding, and staining—was according to Böck (1989).

Calculations of the volumes of the corpora cardiaca (CC) and corpora allata (CA) were carried out. We made micrographs of the serial sections through these glands, drew the outlines of these glands on graph paper with square millimeters, and counted the square millimeters within the outlines (minimal area) and then added the square millimeters that were cut by the outlines (maximal area). The minimal and maximal areas of all sections of a gland were added and multiplied with the thickness of a section (4 μm), taking into account the magnification. By this calculation we obtained minimal and maximal volumes of a gland. The data of the CA volumes given in the Results represent the mean of minimal and maximal volume of a gland ± deviation from maximal and minimal volume. A significant difference between right and left CC or CA exists if minimal and maximal volume of the corresponding glands do not overlap.

RESULTS

Figure 1 shows the habitus of the unilaterally winged specimen of *Lampyris noctiluca*. The appearance is female besides the two somewhat crippled wings on the left side. Normal adult females of *L. noctiluca* are wingless and lack any signs of wing buds during development. The left forewing of the unilaterally winged specimen carried definitively the characteristics of an elytron: heavy sclerotization and lack of veins, and the hindwing that of a wing for flight: membranous structure and venation (Fig. 1). The slightly curved shape of the specimen to the right-hand side was always retained, even when walking.

The genitalia (external sexual appendages) differ distinctly between adult male and female. In the specimen studied here, the genitalia were female (Fig. 2). The luminescent abdominal segments also appeared more female-like, although pigmentation was somewhat stronger, but not to the extent as in males (Fig. 2).

Preparation of the specimen revealed two ovaries with mostly mature and chorionated eggs. But the two ovaries were of different sizes. The ovary of the left, wing-bearing side was much smaller than that of the right side (Fig. 3). The left ovary contained 10, the right ovary 33 mature eggs. Germaria were inconspicuous and immature eggs almost absent in both ovaries, as is normal for adult females. Whether or not the specimen had mated and deposited eggs before capture could not be decided.

The most noticeable deviation from the normal structure of internal organs was an asymmetrical swelling at the left dorsolateral side of the foregut (Figs. 4, 5). The left CC was firmly attached to this growth (Fig. 5). Histological examination of the foregut revealed that the swelling was caused by a strong hypertrophy of the foregut muscles in this area (Fig. 5). The musculature was inconspicuous at other parts of the foregut. The reason for the hypertrophy remains undisclosed. An infection cannot be ruled out, although microbes could not be detected under the light microscope. The CC attached to the swelling showed no pathological alterations (Fig. 4).

Wing development during metamorphosis is commonly under the control of morphogenetic hormones. The absence of JH (i.e., inactivity of CA) is required for the expression of adult features including wings. We therefore analyzed the CA histologically (Fig. 4). Calculations of the volumes of the CA showed significant differences between left and right gland: the left gland measured $221{,}720 \pm 14{,}076\ \mu m^3$ compared to $327{,}520 \pm 22{,}264\ \mu m^3$ of the right gland. Left and right CC were not significantly different in size. Thus, the CA of the wing-bearing side was about one-third smaller than that of the wingless side. PAF-staining of the retrocerebral complex, indicative of neurosecretory products, revealed no differences between left and right glands (Fig. 6).

DISCUSSION

Empirical evidence indicates that there are phenotypic tradeoffs between flight capability and other life history traits in insect females. Females with short or without wings have a higher fecundity and reproduce earlier than those with fully developed wings (Roff and Fairbairn, 1991). Wing dimorphism in Coleoptera is determined by two types of genetic mechanisms, either by single locus systems or polygenic systems. In single locus systems, the short wing trait is typically dominant (Roff, 1986; Dingle, 1991; Roff and Fairbairn, 1991). In Lampyridae, wing dimorphism represents a sexual dimorphism, whereby wing (and flight) reduction occurs in the female to a varying extent, depending on the species (Hess, 1920; Schwalb, 1961). In females of *Lampyris noctiluca,* wings are completely absent. Whereas there is a vast amount of literature on wing polymorphism in relation to migration (see Dingle, 1966), sexual wing dimorphism has received far less attention. In various hemimetabolan insects winglessness at reproductive stages was interpreted as neoteny, i.e., attaining of sexual maturity at a juve-
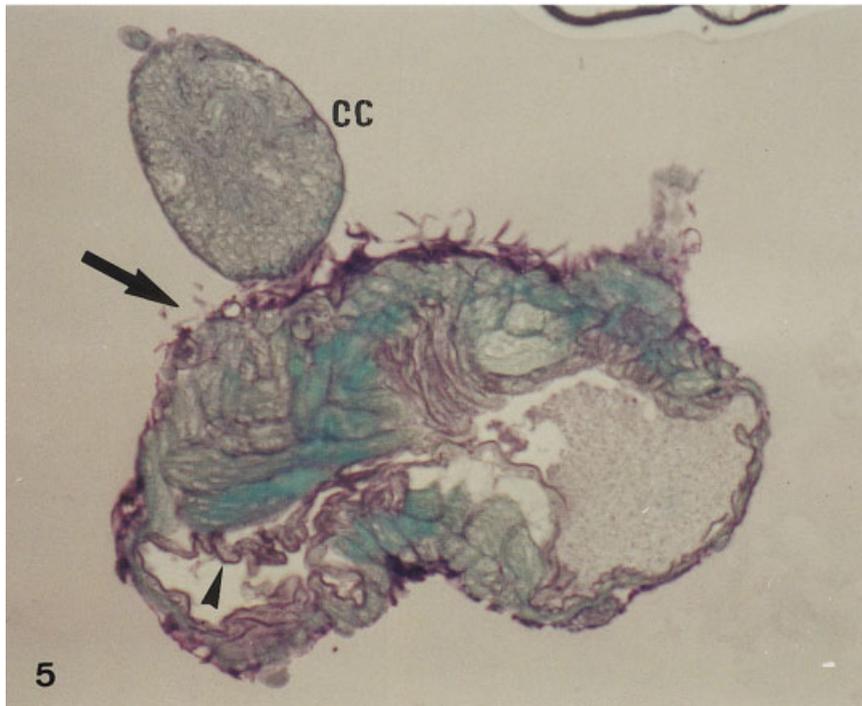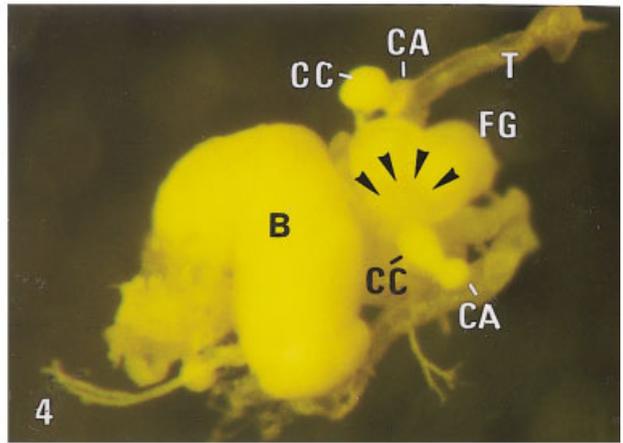
Figures 1–6

nile stage (Hardie and Lees, 1985). This, however, does not apply in the case of *L. noctiluca*. Females undergo pupation and the female beetles, although wingless, present adult characteristics (Naisse, 1966a). The question as to the physiological, i.e., hormonal, control of winglessness has been addressed in Gryllidae (Zera et al., 1989; Cisper et al., 2000) and the planthopper *Nilaparvata lugens* (Ayoade et al., 1996, 1999). In these cases brachypterous and flightless females had larger ovaries that contained a greater number of postvitellogenic eggs compared to macropterous morphs. In *Gryllus firmus* the JH titer was significantly higher in the brachypterous females compared to the macropterous ones (Cisper et al., 2000). Although differences may exist between the species concerning the fine-tuning of wing form determination, brachyptery and aptery are apparently always correlated with an elevation or prolongation of the JH titer at some stage of development, compared to morphs with macroptery (Iwanaga and Tojo, 1986; Ayoade et al., 1996; Zera and Denno, 1997).

Our observations on the unilaterally winged female of *Lampyris noctiluca* is in general agreement with the findings reviewed above. The apterous (right) side of the beetle had a CA that was about one-third larger than that of the winged (left) side; and the ovary of the apterous side included three times more mature eggs than the winged side (33:10). It is, of course, not known when the asymmetrical development of the CA started, and there is no proof that the larger gland produced more hormone than the smaller one. But, in many cases CA activity and CA size are correlated (see Tobe and Stay, 1985). Taken together—ovary size, formation/suppression of wings and CA size—there seems to be enough circumstantial evidence to suggest that the asymmetrical development was caused by a differential JH production in the two sides of the body.

Some intriguing questions remain: 1) What caused the differential expression of right and left CA? The most striking feature of the dissected specimen was the voluminous swelling of the left foregut wall caused by a local hypertrophy of the foregut musculature and the attachment of the left CC to it. How this could affect the CA size (and function), and in consequence wing formation and ovary reduction, remains unclear. One possibility is that the muscle hypertrophy stemmed from an infection that might have also affected the CA, retarding its growth and JH production. 2) Why was JH (apparently) not evenly distributed in the animal by hemolymph circulation? Davydova (1967) mentioned that in her experiments with CA grafting in *Lampyris noctiluca* some manipulated males showed asymmetrical wing formation. The present and Davydova's observations suggest that JH is either short-lived and reaches only close targets, or that circulation is not very effective. 3) Was our specimen probably a genetical gynandromorph? This seems unlikely, since the only "male" characteristic of the specimen was the wings. To present knowledge, wing formation (in contrast to shape and patterning) or its suppression is regulated by morphogenetic hormones that principally occur in both sexes (see above). Gynandromorphs have both male and female morphological features (concerning integumental patterning, sexual appendages, etc.) and tissues resulting from a mosaic of cells containing a male or a female set of chromosomes (Laugé, 1985). But no typical male structures (spare wing formation for the above discussed reasons) were detected here.

According to Naisse (1966a–c), sexual differentiation in *Lampyris noctiluca* is not strictly determined by the genetic status of each cell (as is accepted the case in all other insects), but is governed by an androgenic hormone from the testes, which affects primary and secondary sexual characteristics, including wing formation (see Introduction). The unilaterally winged specimen described here does not support the Naisse hypothesis, since the winged side also contained a regular ovary (although smaller) but no testis, and all other sexual characteristics of the specimen were female.

Fig. 1. This unilaterally winged specimen of *Lampyris noctiluca* was captured in a park-like area in the city of Mainz. The adult beetle exhibits female characteristics except for the two (crippled) wings at the left body side. ×4.7.

Fig. 2. External sexual appendages and lantern of the unilaterally winged specimen (middle) in comparison to a normal female (left) and normal male (right) of *Lampyris noctiluca*. Sexual appendages and lantern of the unilaterally winged specimen are definitely female. ×4.7.

Fig. 3. Dorsal view of the dissected unilaterally winged specimen of *Lampyris noctiluca*. The left ovary (upper side) is considerably smaller than the right ovary (lower side). The eggs are practically all mature and chorionated. ×4.7.

Fig. 4. Brain, retrocerebral complex, and foregut of the unilaterally winged specimen of *Lampyris noctiluca*. The foregut shows a swelling at the left dorsolateral side (arrowheads). The swelling appears somewhat translucent and differs in that from the normal part of the foregut. Note that the lower (i.e., left) corpus cardiacum is attached to this swelling. B, brain; CA, corpus allatum; CC, corpus cardiacum; FG, foregut; T, trachea: ×35.

Fig. 5. Light micrograph of a section through the foregut of the unilaterally winged specimen of *Lampyris noctiluca*. It shows the area of the swelling. The (left) corpus cardiacum (CC) is attached to it, but appears normal. The swelling is caused by a local hypertrophy of the foregut musculature (arrow). The lumen of the foregut is lined by a cuticular intima (arrowhead). ×200.

Fig. 6. Section through the right corpus allatum of the unilaterally winged specimen of *Lampyris noctiluca*. Purple PAF-positive material, indicative of neurosecretions, can be recognized in the parenchyma of the gland. T, trachea, to which the gland is typically attached. ×200.

In conclusion, this extraordinary specimen contradicts the exceptional view of Naisse (1966a) on the regulation of wing dimorphism and sexual differentiation by a male sex hormone. Rather, it supports the current views on the control of these morphogenetic processes.

ACKNOWLEDGMENTS

We thank Dr. Maja Alexenizer for the translation of the article of Davydova from Russian into English. We appreciate the critical review of the manuscript by Dr. Robin Harris, Institute of Zoology, University of Mainz.

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