Eur. J. Entomol. **91**: 85–89, 1994
ISSN 1210–5759

Circadian rhythm of sex pheromone production and male activity of coexisting sibling species of *Cryptomyzus* aphids (Homoptera: Aphididae)

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Aphididae, *Cryptomyzus*, circadian rhythm, sex pheromones, reinforcement, sympatric speciation, mate recognition

Abstract. The aphid sibling species of *Cryptomyzus* live sympatrically on the same host plant and their sexual females appeared to be produced in the same period of the year. The circadian rhythm of the production of their sex pheromones, and the activity of the males, differed between the species. The origin of this divergence of mate recognition and the possibility of speciation by reinforcement are discussed.

INTRODUCTION

The question why mate recognition systems of sibling species often have diverged is widely discussed in evolutionary biology. It has been suggested that divergence may be the result of e.g. sexual selection (West-Eberhard, 1983), a byproduct of allopatric speciation (Paterson, 1985), or the result of reinforcement (Dobzhansky, 1970). If the species co-occur in the same habitat, e.g. herbivorous insects living on the same host plant, they might meet and mate. Then, it depends upon the specificity of their mating systems whether they will interbreed.

The aphid sibling species *C. galeopsidis* (Kaltenbach) and *C. maudamanti* Guldemond reproduce on the same host plant, *Ribes rubrum*, and can be cross-bred, but the hybrids have a reduced fitness (Guldemond, 1990a). They host-alternate and live on different summer hosts, where they only reproduce parthenogenetically. This situation offers an excellent opportunity to study the divergence of mate recognition systems in the absence of spatial isolation. An important question then is whether the sexuals of both sibling species occur in the same period of the year, and whether the daily cycle of production of sex pheromones (Pettersson, 1970) and the activity of the males differ under natural conditions.

MATERIAL AND METHODS

Four populations (10–30 individuals) of *C. galeopsidis* and *C. maudamanti* were collected in The Netherlands in July from their summer hosts *Galeopsis tetrahit* and *Lamium galeobdolon*, respectively. Additionally, one laboratory kept clone of each species was studied. The laboratory clone of *C. galeopsidis* belonged to the red currant host race, and the field collected populations all appeared to be the black currant host race (Guldemond, 1990b). They were cultured from the start of August on their summer hosts in an outdoor insectarium. From mid August the produced autumn females (gynoparae) and males were

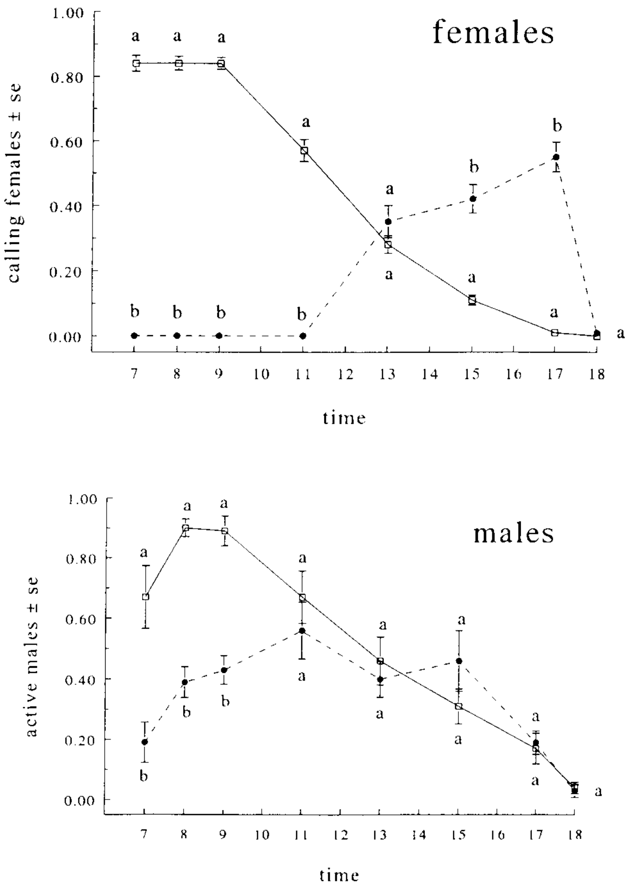

Fig. 1. Phenology of the larvae of sexual females (——) and males (----) of populations of the sibling species of *Cryptomyzus* in 1991. The week that 50% of individuals is produced is indicated.

counted weekly, and the larval sexual females on winter hosts (*Ribes*), which were kept in the cages for one day per week.

The calling behaviour of the sexual females [lifting of abdomen and/or hind tibia, (Pettersson, 1970)] of four populations of each species was assessed on currant plants. Three-5 samples per population of *C. galeopsidis* and 1–3 of *C. maudamanti* were followed for four days. Samples consisted of 10–35 females aged 3–8 and 3–6 days in the two species, respectively. The behaviour of 2–4 days old males was assessed on currant plants in the absence of females. Three populations of each species with one sample per population, consisting of 10–25 males, were followed for three days. The behaviour of the males was classified into "active" (males are walking/searching on plant) and "other" (e.g. feeding). The experiments took place between 7 a.m. and 6 p.m. in the period of 8–11 October. Sunrise was at 6:53/6:58 a.m. and sunset at 6:00/5:53 p.m. Daily minimum and maximum temperatures ranged from 9–13 to 20.5–23°C, respectively. Weather conditions were normal for the time of the year.

The data are presented as the proportion of the individuals that is calling (females) or active (males). For statistical analysis (Anova) the arcsin of the square rooted data were used (Sokal & Rohlf, 1981). To

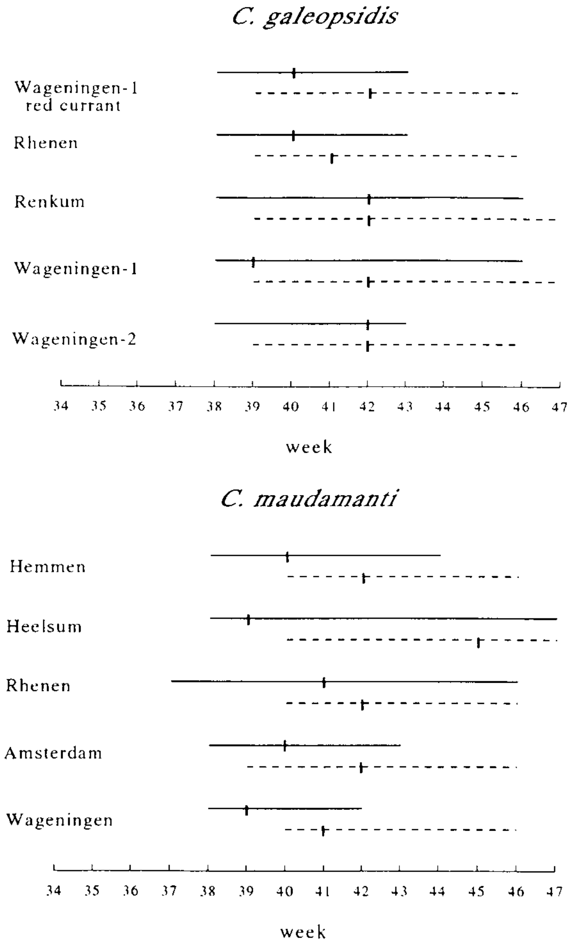

Fig. 2. The proportion of calling females and active males of *C. galeopsidis* (——) and *C. maudamanti* (----) during the photophase.

analyze differences in circadian rhythm between the sibling species Anova's were performed per hour of the day.

RESULTS

The seasonal phenology of sexuals did not differ between the two sibling species of *Cryptomyzus* (Fig. 1, Table 1). The circadian rhythm of the calling behaviour of the sibling species of *Cryptomyzus* differed: *C. galeopsidis* called predominantly in the morning, while *C. maudamanti* had a peak in the late afternoon (Fig. 2). The proportions of calling females differed for all hours (Anova, $P \leq 0.0131$), except for 13:00 and 18:00 hours. No differences in calling were found between conspecific populations, except for the 9:00 and 17:00 hours observations in *C. galeopsidis* (Anova, $P \leq 0.023$). Also, the red currant host race of *C. galeopsidis* showed similar calling times as the three black current host race populations. The activity pattern of the males of *C. galeopsidis* was rather similar to that of its conspecific females, but that of *C. maudamanti* was somewhat less (Fig. 2). Only between 7 and 9 a.m. a significant difference in male activity is found between the species (Fig. 2).

TABLE 1. Mean week number of first, median and last appearance of sexual females and males of 5 populations each of *C. galeopsidis* and *C. maudamanti*.

	first	median	last
C. galeopsidis ♀♀	38.0	40.2	44.2
C. maudamanti	37.8	39.8	44.4
C. galeopsidis ♂♂	39.0	41.2	46.8
C. maudamanti	39.8	42.4	46.2

DISCUSSION

The mate recognition system in the sibling species of *Cryptomyzus* has diverged considerably. Not only differs the daily cycle of production of sex pheromones, but also their specificity (Guldemond & Dixon, 1994). Further, the duration of copulation and the rate of insemination in interspecific matings is smaller compared with intraspecific ones (Guldemond et al., in press; Guldemond, 1992). Because the sexuals of the sibling species have the same phenology, interspecific encounters are possible.

Then, what is the cause of the divergence of their mating systems? Are the differences the result of a random process of genetic change in a period of isolation during speciation? Or are they caused to avoid matings and the production of less fit hybrids, i.e. the result of reinforcement? To answer this question we need to know the mode of speciation of the sibling species of *Cryptomyzus*. If allopatric speciation is likely, the difference in mating system might be the byproduct of the allopatric divergence (Paterson, 1985). In case of sympatric speciation by a host shift, gene flow between the diverging populations (host races) might have taken place. Because aphid host races often have hybrid disadvantage (Mackenzie & Guldemond, in press) individuals that have a somewhat different mating system from the original form might have a selective advantage. This is a situation where speciation by reinforcement is possible.

There is some evidence that the sibling species of *Cryptomyzus* have diverged in sympatry. The summer host of the diverging *C. maudamanti* is found completely in the range of that of *C. galeopsidis*, which indicates that geographic barriers are not likely to have been involved (Guldemond & Dixon, 1994). They fulfil the condition for sympatric speciation, i.e. they are sister species with a sympatric overlap of their host plants (Brooks & McLennan, 1991). This suggests that reinforcement might have caused the differences in mating systems between the sibling *Cryptomyzus* species.

Another possibility might be the process by which individuals with dissimilar daily cycles of sexual activity are less likely to mate and, therefore, can more easily diverge due to assortative mating. This is an analogous process to divergence caused by a difference in the phenology of sexuals (Butlin, 1990).

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