Effect of male densities on sex ratio variations of the predatory gall midge, *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae)

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Abstract
Monogeny, the production of unisexual broods by individual females, is a well-known characteristic in several species of gall midges (Diptera: Cecidomyiidae). Theoretical models have proposed that monogeny may provide a system by which the arrhenogenic/thelygenic females can optionally raise or lower the number of their male/female eggs in response to changes in environmental conditions. In polygynous species, where the males mate with several females, a sex ratio bias toward females is expected to occur when environmental conditions such as food and temperature are suitable. In this paper, first, we evaluated the occurrence and intensity of monogeny in native populations of the polygynous predatory gall midge *Aphidoletes aphidimyza*. Then, we examined the sex ratio variations in relation to different densities of males in the populations. Two proportions of male gall midges (5 and 12 unmated males vs. 10 virgin females) were obtained in plastic cages and the sex ratio of progenies was determined in each density. There was no difference between sex ratio of progenies when the females were exposed to high or low densities of males. Apparently, females cannot regulate the number of female-producing and/or male-producing progenies in response to male densities. Our results incline us to think about other benefits that may have been achieved through transition to monogeny.

Keywords *Aphidoletes aphidimyza*; Cecidomyiidae; gall midges; monogeny; sex ratio regulation.

1 Introduction
The family Cecidomyiidae is one of the largest groups of flies (order: Diptera) whose members are commonly known as gall midges (Cilbircioglu and Unal, 2009). An unusual reproductive characteristic that has been found in some species of gall midges is that monogeny in which, all offspring of an individual female are either exclusively male or exclusively female (Tabadkani et al., 2011). In this system, a single maternal-effect autosomal gene (called chromosome maintenance, Cm) prevents the elimination of paternally derived X chromosome during early stages of embryogenesis, so that all of the offspring of Cm-bearing mothers obtain a female-determining karyotype. The absence of Cm usually has the opposite effect; all of the offspring of Cm-lacking females lose the two paternally derived X chromosomes and obtain a male-determining karyotype (Stuart and Hatchett, 1991; Benatti et al., 2010).

Theoretical aspects of sex determination in monogenous gall midges predict an equal number of female-producing and male-producing females within populations (see Benatti et al., 2010) such that the overall population sex ratio is 1:1 (empirical data include Sell, 1976; Baxendale and Teetes, 1981; Stuart and Hatchett, 1991; Benatti et al., 2010).
1991; Dorchin and Freidberg, 2004). However, observations of some strictly monogenous populations with skewed sex ratio (for examples *C. sonchi* (Bremi) (McClay, 1996), *Rabdophaga heterobia* Loew, *Izeniola obesula* Dorchin (Dorchin et al., 2007), and *Aphidoletes aphidimyza* Rondani (Gilkeson and Hill, 1986; Havelka and Zemek, 1999) have raised the question of whether gall midges are able to adjust the sex ratio of their offspring in response to changes in environmental conditions.

Manipulation of offspring sex ratio enables arthropods that possess this ability to quickly respond to changes in environmental conditions and so, increase the overall fitness of their population (Dorchin and Freidberg, 2004). In protandrous insects such as gall midges (Mo and Liu 2007; Ogah et al., 2010), when males are polygynous (mate with several females) and females are monandrous (mate with a single male), theory predicts a male-biased sex ratio because the males that emerge earlier than females have the greatest opportunity to get virgin mates (Muralimohan and Srinivasas, 2010). However, in some species of gall midges such as *A. aphidimyza*, it has been shown that excessive numbers of males lead to interrupted mating and to females unable to reproduce (van Lenteren and Schettino, 2003). So, the question is that how female gall midges respond to increased numbers of males in their population. In a simple expression, Monogeny may provide an opportunity for sex ratio regulation in gall midges (Barnes, 1931; Harris et al., 2003) if female-producing and male-producing females can oviposit differently in response to environmental factors. Based on this hypothesis, in this study, we evaluated sex ratio variation of the predatory gall midge *A. aphidimyza* in response to various densities of males in the population. *A. aphidimyza* is a monogenous species (Sell, 1976) that has been successfully used for biological control of several aphid species especially in greenhouses (Choi et al., 2004). Although, the occurrence of monogeny has been approved in Russian populations of *A. aphidimyza* (Sell, 1976), we did an experiment to determine the occurrence and intensity of monogeny in natural populations of this species native to Iran.

2 Materials and Methods
2.1 Plants and insects
In order to rear the host aphid, *Aphis fabae* (Hemiptera: Aphididae), broad bean seeds, *Vicia fabae* (Fabaceae) were planted in plastic pots (20 cm height and 16 cm diameter) containing a mixture of sawdust. The aphids were obtained from Zanjan province (middle of Iran) and at the time of this study, they had been reared in laboratory conditions for at least three years. *A. aphidimyza* were gathered as larval stage from Bojnurd (east of Iran). The stock colony of *A. aphidimyza* was constituted in cages (1m × 1m × 90 cm) with four net walls, a wooden bottom, and a glass ceiling. Several unknown spiders were released in the cages for a few hours until they had made several webs. After adult emergence, some broad bean pots, infected by *A. fabae*, were introduced to the cages and the mated females started oviposition immediately. The cages were kept in a small growth chamber with a temperature of 24 ± 3 °C, 65-75% relative humidity, and a photoperiod of L16-D8.

2.2 Sex ratio of stock colony of *A. aphidimyza*
According to our personal observations, male *A. aphidimyza* emerge up to one day faster than females. Therefore, two days after the emergence of adult gall midges, 340 individuals were captured at random and their sex was determined by considering their antenna form. The males have longer hairy antenna, while the females’ antenna is shorter without long hairs. The sex ratio of stock colony was determined by dividing the number of males to all counted individuals.

2.3 Occurrence and intensity of monogeny
To determine the occurrence and intensity of monogeny in Iranian populations of *A. aphidimyza*, 22 ovipositing gall midges were captured at random from the stock colony and released separately in cylindrical plastic cages (20 cm diameter, 30 cm height) containing some bean plants infected by *A. fabae*. The females
were allowed to oviposit for two days and after it, the plants were transferred to smaller new boxes (10 cm diameter, 15 cm height). The larvae were provided by *A. fabae* daily. After adult emergence, their sex was determined as previously was said.

### 2.4 Sex ratio variation of *A. aphidimyza* in response to male density

In this experiment, the gall midges were separately transferred to small plastic cans in larval stage, where we could see when the adults emerge. After adult emergence, two proportions of male to female gall midges (5 males vs. 10 females as low density and 12 males vs. 10 females as high density) were obtained. Some broad bean plants infected by *A. fabae* were separately placed under cylindrical plastic cages (30 cm diameter and 40 cm height) and the prepared proportions of male and female gall midges were released separately in the cages. A Kleenex was soaked in sugar solution (10%) and expanded in each cage for gall midges nutrition. After emergence of adults in F1, a maximum number of 235 (but often less) gall midges were randomly captured from each container and their sex was determined. The sex ratio of offspring was calculated as previously was said. This experiment was carried out in 10 replications. Data analysis was carried out by use of SPSS computer software version 15.

### 3 Results

#### 3.1 Sex ratio of stock colony of *A. aphidimyza*

The sex ratio of native gall midges’ populations was slightly male biased (54.7% males vs. 45.3% females). Out of 340 adult gall midges that were sampled directly from the stock colony, 186 and 154 were males and females, respectively. Previous studies on *A. aphidimyza* also showed a male biased sex ratio in colonies that had been obtained from commercial companies (Gilkeson and Hill, 1986; Heimpel and Lundgren, 2000; Tabadkani et al., 2012a). Interestingly, shortly after adult emergence, the sex ratio of progenies biases toward females as a result of differential mortality among sexes.

<table>
<thead>
<tr>
<th>Rep</th>
<th>Arrhenogenic females</th>
<th>Thelygenic females</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>16</td>
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<tr>
<td>4</td>
<td>9</td>
<td>31</td>
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<tr>
<td>5</td>
<td>8</td>
<td>18</td>
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<td>6</td>
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<td>11</td>
<td>28</td>
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</tr>
<tr>
<td>12</td>
<td>14</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>155</td>
</tr>
</tbody>
</table>

#### 3.2 Occurrence and intensity of monogeny

Iranian populations of *A. aphidimyza* show strict monogeny, all of the 22 females produced progenies of the same sex. The numbers of female-producing and male-producing gall midges were 10 and 12 respectively (Table 1). This also confirms occurrence of slightly male-biased sex ratio of *A. aphidimyza* at emergence time.
(53.7% males), however we did not calculate the true number of eggs deposited by female-producing and male-producing females.

3.3 Sex ratio variation of *A. aphidimyza* in response to male density

In this experiment, there was no significant difference between the sex ratio of *A. aphidimyza* populations in two densities of male gall midges (t-test: t = 1.17, d.f = 18, P>0.05). The sex ratio of adult gall midges varied from 53.2% to 58.1% and from 52.8% to 57.2% in low densities and high densities of males, respectively (Table 2, Fig 1). In the replications in which the number of individuals did not reach 235, we calculated sex ratio by counting all of the progenies present in the cages. Although the sex ratios of gall midges biased slightly toward males in the first generation (55.63 and 54.81 in low and high density of males, respectively), they showed no significant difference with the sex ratio of stock colony (54.7) (one-sample t-test, d.f=10, t=1.69 in low male density and t=0.254 in high male density, P>0.05).

Table 2 Sex ratio (proportion of males in F1 progenies) of *Aphidoletes aphidimyza* determined in two different densities of males

<table>
<thead>
<tr>
<th>Rep</th>
<th>Low density of males</th>
<th>High density of males</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>96</td>
</tr>
<tr>
<td>2</td>
<td>122</td>
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<tr>
<td>3</td>
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<td>95</td>
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<td>9</td>
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<td>10</td>
<td>118</td>
<td>95</td>
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<tr>
<td></td>
<td>Average</td>
<td></td>
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</tbody>
</table>

Fig. 1 Sex ratio variations of *Aphidoletes aphidimyza* in relation to two different densities of adult males in the population, a comparison has been done between the two densities showed by horizontal line. The lowercase letters show significant difference between the two densities with the sex ratio of the stock colony.
4 Discussion

Although, the occurrence of female biased sex ratio has been repeatedly reported in various species of gall midges (Baxendale and Teetes, 1981; Matuszewski, 1982; McClay, 1996; Dorchin and Freidberg, 2004; Smith et al., 2004; Mo and Liu, 2007; Ogah et al., 2010), studies on A. aphidimyza have been suggested that this species has a slightly male biased sex ratio in both laboratory and field conditions (Gilkeson and Hill, 1986; Heimpel and Lundgren, 2000; Tabadkani et al., 2012a; current study, see Fig. 2). There are evidence that estimated female-biased sex ratio in most species of monogenous gall midges is associated with differential mortality of male and female progenies under harsh conditions (Dorchin and Freidberg, 2004; Smith et al., 2004; Dorchin et al., 2007; Tabadkani et al., 2012b). For example, the sex ratio of the wheat midge Sitodiplosis mosellana biases toward females by 6-11% as a result of differential mortality during diapause (Smith et al., 2004). Similarly, we have shown previously that the sex ratio of A. aphidimyza is estimated female-biased because of early elimination of males from the natural populations (Tabadkani et al., 2012b). Apart from that, the role of the feminizing strains of the endosymbiotic rickettsia bacteria belonging to the type species, Wolbachia pipientis in sex determination and sex ratio distortion is yet to be clearly understood (Omoloye, 2006). Already, W. pipientis has been found only in females of the Asian rice gall midge (Behura et al., 2001) and its absence in male gall midges questions whether they have a role in sex determination during early embryogenesis.

Iranian populations of A. aphidimyza show strict monogeny like some other species of gall midges (see Tabadkani et al., 2011). Monogeny in gall midges has been proposed to enable the females to adjust their offspring sex ratio in response to environmental conditions (Bull, 1983). However, results of this study along with those of previous works (Dorchin and Freidberg, 2004; Tabadkani et al., 2012a) suggest that female gall midges do not have any control on their population sex ratio. In our experiment, there was no significant difference between the sex ratio of A. aphidimyza in low and high densities of males. Our previous study also showed inability of female A. aphidimyza to adjust their offspring sex ratio in response to host density (Tabadkani et al., 2012a), an important factor that have been well studied in haplodiploid insects (Craig et al., 1992; Morrill et al., 2000; Ode and Heinz, 2002; Kishani Farahani et al., 2011) and some parasitic flies (Morrison et al., 1999). Studying on the gall midge I. obesula, Dorchin and Freidberg (2004) found that the sex ratio among the galls of this species fluctuated throughout the year, dropping to a minimum of 20.5% male galls in spring and rising to a peak of 50% male galls in winter. However, they believed that these fluctuations are only a consequence of differential mortality of male and female progenies during summer (Dorchin and Freidberg, 2004).

Sex ratio regulation in monogenous gall midges is sometimes compared with that of haplodiploid insects in the order Hymenoptera. However, it should be kept in mind that these two groups have different patterns of sex allocation. In a simple expression, inability of monogenous gall midges to regulate their offspring sex ratio is easily justifiable. Because female gall midges exclusively produce unisexual broods, the fitness of each individual is not associated with her offspring sex ratio (Dorchin and Freidberg, 2004), so each female will try to elevate her fitness by maximization of her offspring frequency regardless of their sex. Therefore, an arrhenogenic gall midge does not reduce their progenies in favour of her thelygenic sisters when environmental conditions are suitable. On the other hand, since haplodiploid insects produce both male and female progenies, they easily regulate their offspring sex ratio in response to environmental conditions.

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