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Research Article

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Sex allocation and field population sex ratio of *Apanteles taragamae* Viereck (Hymenoptera: Braconidae), a larval parasitoid of the cucumber moth *Diaphania indica* Saunders (Lepidoptera: Crambidae)

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Abstract: Sex ratio is one of the most important biological characteristics of arthropods. In a parasitoid population, sex ratio can influence the suppression of host populations or the stability of host–parasitoid interactions in the field. In this study, a survey was carried out to determine the sex allocation through the sequence of male/female adult emergence and calculate the sex ratio of selected populations of *Apanteles taragamae* in their natural habitat. Assessment of sex ratio at the population and individual level (brood size per female) was examined. We found no difference in the likelihood that either sex would emerge before the other. Observations of sex ratio at the population and individual level reveal a females biased sex ratio. Analysis of the relationship between brood size and sex ratio of *A. taragamae* shows that brood size may influence the proportion of male to female offspring yielded. A male-biased sex ratio tends to be found in smaller brood size. However, sex ratio is shifted to female biased in larger brood size.

Keywords: *Apanteles taragamae*, biological control, brood size, host–parasitoid interaction, population structure

1 Introduction

Sex allocation is the allocation of energy or resources to male versus female offspring within a given population and is a characteristic of sexually reproducing species. In aggregate, sex allocation forms a sex ratio in the population. If males and females are equally costly to produce, then sex allocation collapses to be the same as the sex ratio produced; however, one can have equal sex allocation and unequal sex ratios [1]. Ideally, the sex ratio of most species that produce offspring through sexual reproduction is approximately 1:1 between males and females [2]. However, female-biased sex ratios are often found in gregarious parasitoid populations under local mate competition [3] as the main factor besides resources quality, partial bivoltinism, and presence of constrained females [4]. It is common to see variation in the sex ratio of hymenopteran parasitoids ranging from 100% males due to unmated females, to female-biased ratios, up to a 100% female sex ratio due to maternally inherited symbionts [5–7]. Hymenopteran parasitoids can easily experience skewed sex ratio as a result of arrhenotokous parthenogenesis, a mode of reproduction where unmated females will produce only male progeny, while mated females can produce both female and male progenies [8]. The option of arrhenotokous parthenogenesis, and the mating condition of females (mated or unmated) before they disperse are two important factors that influence the field sex ratio in a population of hymenopteran parasitoids. The higher the proportion of virgin females, the higher numbers of males that are going to be produced, hence skewing the sex ratio to male bias in the population.

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Sex determination in parasitoids can be controlled by genetic factors [9,10] or affected by external causes, such as host quality [11], host density [12,13], number of founder females [14,15], and also superparasitism [16,17]. Ultimately, choices made by females to oviposit male or female eggs during oviposition will determine the sex ratio of its offspring [18,19]. Sex ratio in the field is affected by the sex-determining mechanism and the numbers of virgin females. The population sex ratio is an important parameter that should be well understood, especially for biological control purposes, since it is mostly females that will determine the success of pest control.

Various studies on parasitoid sex ratio have been conducted. However, most are lab-based [5], and only a few reports are available on parasitoid sex ratio in the field; for example, [20–24]. In all these research, a female-biased sex ratio was found in the fields. Sex ratio of *Epidinocarsis lopezi* in the field tends to shift into more male-biased under high density [25], while the sex ratio of all three species of *Adialytus arvicola*, *Lysiphlebus hirticornis* and *Puesia pini* at eclosion were female-biased and did not vary with brood size [20]. Moreover, most prior research has considered sex ratio in parasitoids only for eggs or pupae [26]. There are still many unanswered questions regarding parasitoid behaviors that affect the sex ratio of wild populations, such as, do females mate before they disperse to seek hosts? Can mating occur after female parasitoids fly from their natal locale? These are amongst the most important questions in behavioral ecology of parasitoids since mating status might affect the sex ratio – and hence the stability – of populations in the field. To investigate these questions, we conducted a study using *Apanteles taragamae* (Hymenoptera: Braconidae), a primary larval endoparasitoid of the cucumber moth *Diaphania indica* [27,28].

Apanteles taragamae holds promise as a biological control agent of *D. indica*. *A. taragamae* demonstrate an 86% parasitism rate on *D. indica* in the lab [29]. However, programs to utilize *A. taragamae* for pest management purposes may be limited by the difficulties in mass-rearing the parasitoid in captivity. For mass-rearing a preponderance of females is preferable, but it is difficult to achieve in the lab [30]. Because sex ratio is an important indicator for the sustainability of a wild population, and the proportion between male and female progeny will determine the rigor of the population, it is best to ensure a balanced male–female ratio. Unmated females (virgin females) that disperse to seek hosts will produce 100% male offspring in the new locale (thereby limiting future reproduction in the population), whereas females who mate prior to dispersal are able to control sex ratio of their offspring based on the quality of hosts, and the presence of other females in each area. Therefore, it is important to understand the copulation status (virginity versus mated) of females in the field because this information can help decode the complex behavior of parasitoids.

The present study addresses the following questions: (1) How common are unmated females ovipositing in the wild? (2) What is the field sex ratio of *A. taragamae* in the study area? To address these questions, we assess the field sex ratio of a larval parasitoid of the cucumber moth *D. indica* in four different populations.

2 Materials and methods

2.1 Research location

Research was carried out in Kecamatan Dramaga, Desa Dramaga (6°32′57.57″S 106°44′33.32″E) and Desa Cikarawang (6°32′56.13″S 106°43′39.63″E) in 2014 and Kecamatan Ciampea, Desa Cihideung1 (6°35′14.83″S 106°43′43.73″E) and Desa Cihideung2 (6°35′18.77″S 106°43′01.31″E), Kabupaten Bogor, Jawa Barat, Indonesia, in 2015. These locations were selected based on the proximity of the appropriate cucumber fields area (25 m × 15 m), easily accessible from the research lab location.

2.2 Insect sampling

Collections were conducted on both *A. taragamae* cocoon clusters and the parasitized larvae of *D. indica*. A 25 m × 15 m cucumber plots were used in the sampling. Each plot was divided into 4 subplots and all plants within the subplots were sampled systematically using the same method as previous research [31]. Sampling was done directly by collecting insects found in selected plants during a 3-week period. Samplings were carried out weekly in the same plots each week, which began when plants were 3 weeks old until 5 weeks old (the time at which farmers applied pesticides to the field). These insects were then reared in laboratory conditions (25 ± 1°C, 90 ± 10% RH and L16:D8 photoperiod). Parasitized *D. indica* larvae were reared in a transparent plastic cage (15 cm in length, 10 cm in width, and 5 cm in height), until the formation of the parasitoid's cocoon. The *D. indica* larvae were fed a natural diet of cucumber leaves. Once formed, the parasitoid cocoon cluster of the parasitoid was placed in a cylindrical glass tube (1.5 cm in diameter, 10 cm in length), with honey droplets provided as a food source. After the emergence of the adult parasitoid, the sex ratio of the progeny was recorded, and the number of cocoons per-cluster was calculated.

2.3 Sex allocation of *A. taragamae*

Sex allocation (individual emergence of *A. taragamae*) was measured based on direct observation of the emergent adults collected from 30 cocoon cluster colonies which were randomly selected from all collected samples from all research locations. Each cocoon cluster was placed in a cylindrical glass tube (1.5 cm in diameter, 10 cm in length). The emergence pattern of adult parasitoids was measured in terms of the order of and sex of adult parasitoids as they emerged from the cocoon, recorded first, second, and so on, through all emerged adults from a cocoon cluster. We assume that there was no superparasitism in any observed cocoon cluster.

2.4 Sex ratio of *A. taragamae*

Since *A. taragamae* is a gregarious parasitoid, it is possible to conduct a sex ratio analysis at the population and individual (parental) level. Population sex ratio is the overall sex ratio of *A. taragamae* at a given site/locality, whereas individual sex ratio is the ratio of male/female within a single host. Since *A. taragamae* is a gregarious parasitoid, it is possible to measure the sex ratio within a host, which depicts the brood size. Sex ratio was assessed at the individual level by assessing the relationship between brood size to the sex ratio of *A. taragamae*. At the individual level, sex ratio was used to determine copulation status of *A. taragamae*, by calculating the sex ratio of parasitoid progeny produced by one host larva from each population (sex ratio per-host).

At the population level, the parasitism rate, the survival rate, and sex ratio of each population were measured. Finally, the relationship between brood size and sex ratio for *A. taragamae* was calculated using a modification of the method described by Peter and David [28]. Sex ratio was determined based on the brood size of 1–5, 6–10, 11–15, 16–20, and 20–25 progeny/host. Unmated females were measured from 100% males emerging from each population data.

2.5 Statistical analysis

The sex allocation of *A. taragamae* was analyzed using chi-square analysis using Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc.). General Linear Model (GLM) analysis was used to analyze the performance of *A. taragamae* in the field such as parasitism and the survival rate in all research locations. Parasitism rate was calculated based on the number of parasitized hosts divided by all host larvae taken from the field, while the survival rate was calculated based on the number of emerging adults of *A. taragamae* divided by the total number of cocoons formed per host. A GLM with the binomial family using two factors, i.e., research location and parasitoid brood size were used to analyze sex ratio of *A. taragamae*. The overall results of the analysis that were significantly different were further tested using the HSD Tukey test at a 95% level of significance. Data analysis was carried out using R v 4.0.3 software [32] and visualized using the ggplot2 package [33].

3 Results

3.1 Sex allocation of *A. taragamae*

The sex ratio of parasitoid adults counted during eclosion from cocoon clusters varied (Appendix Table 1). There was no difference in the likelihood that either sex would emerge before the other ($\chi^2 = 3.056$, $P = 0.549$). In some cases, males and females appear alternately. There are also males or females that appear 2 to 5 times in a row and then follow by the other sexes. Overall, 65.5% of adults observed emerging first from cocoon clusters were female, with the rest male. Two cocoon clusters produced male or female progenies only.

3.2 Performance and the sex ratio of *A. taragamae* in the field

Our study showed that there was a significant difference in parasitism rate of *A. taragamae* between research locations (GLM: $F_{3.43} = 14.511$, $P < 0.001$). Parasitism rate of *A. taragamae* in Cihideung2 was higher than that of Cihideung1, Cikarawang, and Dramaga which had the same level of parasitism rate (Figure 1). In contrast, the survival rate of *A. taragamae* did not have a significant difference between populations in the four cucumber planting sites (GLM: $F_{3.43} = 1.346$, $P = 0.272$). The survival rate of *A. taragamae* in this study ranged from 4.34 to 100%.

Interestingly, there is no significant difference in sex ratio of *A. taragamae* at population level in all research locations ($F_{3,422} = 0.95$, $P = 0.412$). The entire population

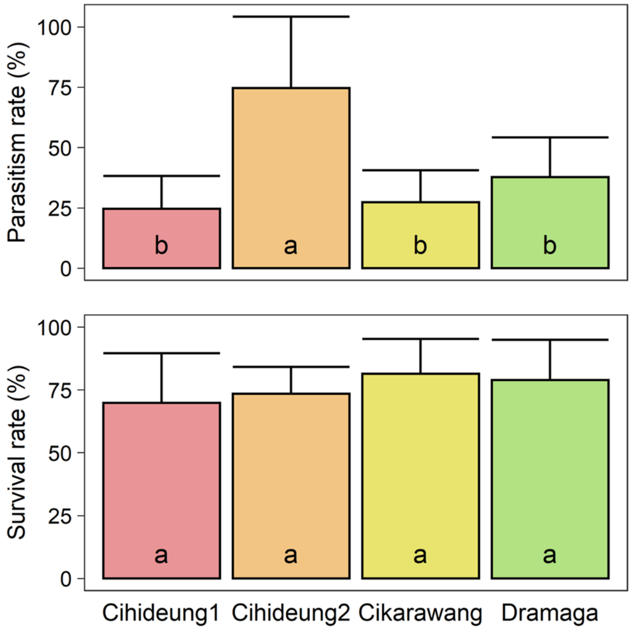

Figure 1: Parasitism and survival rate of *Apanteles taragamae* in all research locations. Means with different letter are significantly different by Tukey HSD Test (α = 0.05).

of *A. taragamae* showed a tendency to female biased (Figure 2) sex ratio in population level. Similar pattern is also found in sex ratio at individual level which is observed by the number of brood size. We found that the sex ratio was different either on different locations ($F_{12,410}$ = 2.046, P = 0.01) or different brood size ($F_{4,429}$ = 10.485, $P < 0.001$). The brood size produced by *A. taragamae* ranged from 1 to 25. We found a male-biased sex ratio of *A. taragamae* emerging from smallest brood size [1–5] in all research locations, but the offspring sex ratio would be female-biased at larger brood size [6–20]. The highest frequency of female-biased sex ratio of *A. taragamae* is found in 11–15 brood size. We found 1:1 sex ratio of *A. taragamae* at 21–25 brood size (Figure 3) in 3 different research locations.

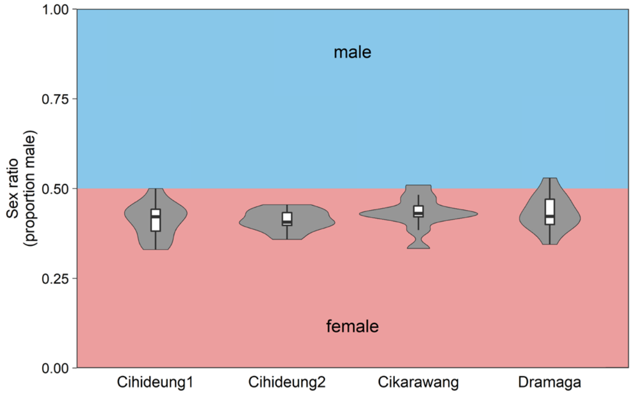

Figure 2: Sex ratio of *Apanteles taragamae* at the population level in all research locations.

A sex ratio of 100% female or male was also found. 100% female progenies were found in a low proportion (0–12%) of cocoon sampled in each location. Meanwhile, 100% male progenies were found as many as 8–16% in each location (Figure 4). 100% male progenies found from emerging adult parasitoids with 4.34–50% survival rate (N = 45), and 100% female progenies was found from that of 5.55–90.9% (N = 25) survival rate.

4 Discussion

The biological characters of sex allocation and sex ratio are interrelated in parasitoid biology together forming an influential factor in population outcomes. Sex allocation by female parasitoids can be discerned by observing oviposition sequences which are evident eventually in progeny sex ratio. Sex allocation of *A. taragamae* as observed through their adult emergence pattern show no tendency to oviposit male or female progeny either at the beginning or at the end of her oviposition sequences. The same mechanism of oviposition sex allocation is found in *Cotesia glomerata* [34]. For that species, the female parasitoid generally follows a strategy of first depositing a male egg at the beginning of oviposition sequence. Similarly, *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) will oviposit male eggs first in sequence [35]. This “first male” strategy has also been seen in *T. basalis* (Hymenoptera: Trichogrammatidae) [36], *Telenomus busseolae* (Hymenoptera: Scelionidae) [37], *Psyttalia concolor* (Hymenoptera: Braconidae) [38], and *Anaphes nitens* (Hymenoptera: Mymaridae) [39]. The first male strategy may relate to the precopulatory mate-guarding behavior that is common in some animals including hymenopteran insects [40]. However, recent study on the sex allocation of *Nasonia vitripennis* showed that the male develops faster than female embryo causing male to emerge first before female [41].

Individual sex ratio (sex ratio per-host) for hosts in the field for each study area – or patch – which is described by the brood size showed an interesting result, with male-biased broods being present in smaller brood size, and female-biased broods in larger brood size. The differences in sex ratio at each brood size may be influenced by the host quality. Brood size with a female-dominant sex ratio presumably results from high quality host conditions, and vice versa. Godfray [5] concurs that the parasitoid sex ratio is affected by host quality and size.

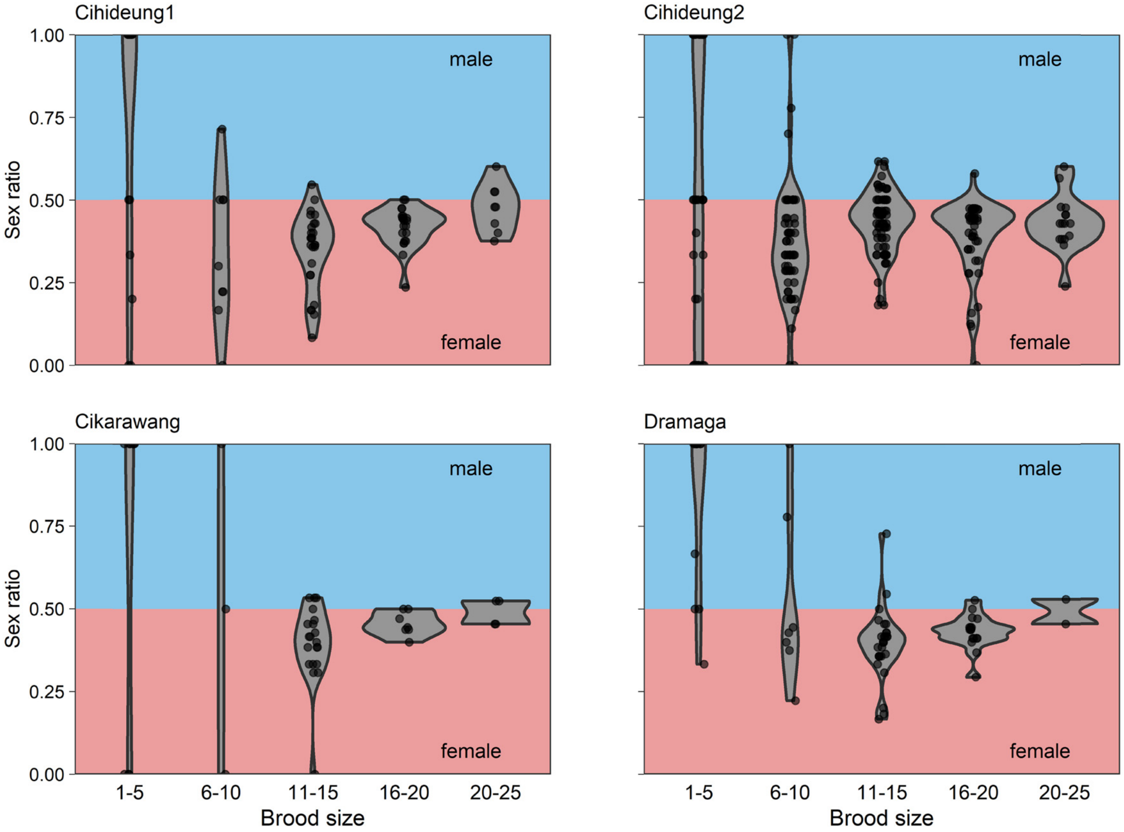

Figure 3: Relationship between brood size and sex ratio of *Apanteles taragamae* in all research locations. The black dot representing the sex ratio obtained for each observed brood size.

Although we did not include the effect of host quality in this study, based on other research findings it is known that brood size has a positive correlation with the host quality. Female parasitoids will lay fewer eggs in lower-quality hosts, and these eggs will develop as male progeny. Conversely, the female will lay more eggs in higher-quality hosts, and these eggs will develop as female progeny because female need more nutrients than male [42]. The effect of host quality on parasitoid sex ratio has been demonstrated in other parasitoids, for example [43–45]. The relationship between brood size and sex ratio in response to host quality was also reported by Li et al. [46] in a gregarious parasitoid *Oomyzus sokolowskii* (Hymenoptera: Eulophidae).

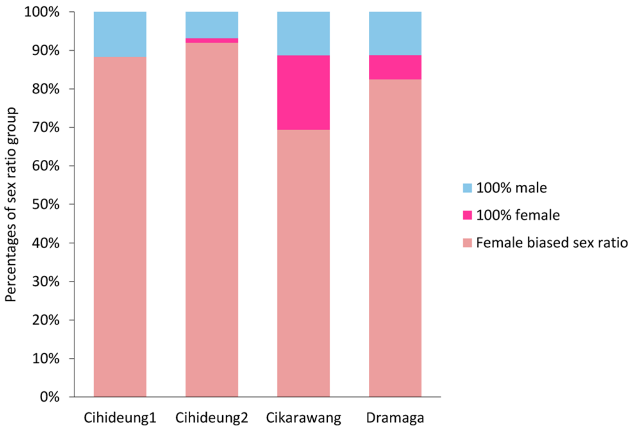

Figure 4: Proportion of sex ratio of *Apanteles taragamae* in all research locations.

In addition, the presence of 100% female sex ratio which emerge is of considerable interest because for biological control practices a female-biased sex ratio for parasitoid species is beneficial, because a larger proportion of females will ensure future generations, and the sustainability of the parasitoid population in the field is enhanced. Female parasitoids also drive parasitism of pest hosts via oviposition thus decreasing pest populations. However, 100% female sex ratio is rarely found. Only 5.76% of population has a 100% female sex ratio. Our study indicates that most females that oviposits have copulate, but whether the copulation occurs prior to or after dispersal remains unknown. If it is assumed that mating after dispersal may decrease the probability of finding a mate, then is it very possible that females may already copulate prior to dispersal from their natal habitat. We also found a 10.36% of populations having 100% male sex ratio. This result means that there are unmated/virgin females in the field (assuming that all females have not being depleted of their sperm and assuming there are no extrachromosal factors that affect the sexes of the offspring) [47,48]. However, what is the frequency of these unmated females remains to be seen. Although female predominant populations are beneficial, a male biased sex ratio (at the brood level) may help the population increase their genetic variability. Our results can be used as the basis for more comprehensive work looking at the effect of mating behavior and other factors that may influence *A. taragamae* sex ratio. Previous studies have shown the effective host searching ability of *A. taragamae* [49,50] and high parasitism rate [30,51] thus rendering *A. taragamae* as a promising biocontrol agent in the future. These factors are shown to be important in influencing the suppression of host populations and/or the stability of host–parasitoid interaction in the field [52].

In summary, analysis of the sequence of individual emergence (sex allocation) showed that there is no difference in the likelihood that either sex would emerge before the other, indicating that there is no tendency whether male or female was preferred to be oviposited before the other. Observations of sex ratio reveal a similar pattern that the sex ratio of *A. taragamae* is females biased either at the population and individual level, indicating that most females have already copulated prior to dispersal from their natal location, and unmated females are rarely found in the field. Sex determination in *A. taragamae* is affected by external factors such as host quality which may be reflected by the relationship between brood size and sex ratio.

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Appendix

Table 1: Adult emergence pattern of *Apanteles taragamae* Viereck (Hymenoptera: Braconidae)

No. of observed cocoon cluster	Sequence of adult emergence pattern																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	♂	♀	♂	♀	♀	♀	♂	♀	♂	♀	♂	♀										
2	♂	♂	♂	♂	♂	♂	♀	♀	♂	♂	♀	♂										
3	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♂	♀	♂	♀	♀							
4	♀	♀	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀							
5	♀	♀	♀	♀	♀	♀	♀	♂	♀	♂	♀											
6	♂	♂	♂	♂	♂	♀	♂	♂	♂	♂	♀	♂	♂	♂								
7	♀	♀	♀	♂	♂	♂	♀	♀	♀	♀	♀	♀	♂	♀	♀	♀						
8	♀	♀	♀	♀	♂	♂	♂	♂	♂	♂	♂	♀	♀									
9	♀	♂	♂	♀	♂	♂	♂	♂	♂	♀												
10	♀	♀	♀	♀	♂	♀	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂						
11	♂	♀	♂	♀	♂	♀	♀	♂	♂	♀	♂	♂	♂	♂	♂	♀						
12	♀	♀	♀	♀	♀	♀	♀	♀	♀	♂	♂	♂	♂	♂	♀	♂						
13	♂	♀	♀	♂	♀																	
14	♀	♀	♀	♀	♀	♀	♀	♀														
15	♀	♀	♀	♂	♀	♂	♀	♂	♂	♂	♀	♂	♂	♀								
16	♀	♀	♂	♀	♀	♀	♀	♂	♂	♀	♀	♀	♂	♀	♀	♀	♂	♀	♀	♀		
17	♀	♀	♀	♂	♂	♂	♂															
18	♂	♂	♂	♀	♂	♂	♂	♂														
19	♀	♀	♀	♀	♀	♀																
20	♂	♀	♂	♀	♀	♀	♂															
21	♂	♀	♀	♀	♂	♀	♀	♀														
22	♂	♂	♂	♀	♀	♂	♀	♀														
23	♂	♂	♂	♂	♂	♂	♂	♂	♂	♀	♀											
24	♀	♀	♂	♂	♀	♀	♀	♀	♂	♂	♂	♂	♂	♂	♂							
25	♀	♂	♂	♀	♂	♂	♂	♀	♂	♀	♂	♀	♀	♀	♀	♀	♂	♀	♂	♂	♂	♂
26	♀	♀	♀	♀	♂	♀	♀	♀	♀	♀												
27	♀	♀	♂	♂	♀	♀	♀	♀	♂													
28	♀	♀	♀	♀	♀	♀	♀	♀														
29	♀	♂	♀	♀	♂	♂	♀	♀	♀	♂	♀	♂	♀	♀	♀	♀	♀	♀	♂	♀		

♂: male, ♀: female.