



Title	Frequency-dependent selection acting on the widely fluctuating sex ratio of the aphid <i>Prociphilus oriens</i>
Author(s)	Li, Y.; Akimoto, S.
Citation	Journal of evolutionary biology, 30(7), 1347-1360 https://doi.org/10.1111/jeb.13107
Issue Date	2017-07
Doc URL	http://hdl.handle.net/2115/70896
Rights	This is the peer reviewed version of the following article: Journal of evolutionary biology:30(7):1347-1360, 2017 July, which has been published in final form at https://doi.org/10.1111/jeb.13107 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	document Re 3.8.pdf



[Instructions for use](#)

1 Title: **Frequency-dependent selection acting on the widely fluctuating sex ratio of**
2 **the aphid *Prociphilus oriens***

3

4

5 Running title: Frequency-dependent selection

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31 **Abstract**

32 Frequency-dependent selection is a fundamental principle of adaptive sex-ratio
33 evolution in all sex ratio theories but has rarely been detected in the wild. Through
34 long-term censuses, we confirmed large fluctuations in the population sex ratio of the
35 aphid *Prociphilus oriens* and detected frequency-dependent selection acting on these
36 fluctuations. Fluctuations in the population sex ratio were partly attributable to climatic
37 factors during the growing season. Climatic factors likely affected the growth
38 conditions of host plants, which in turn led to yearly fluctuations in maternal conditions
39 and sex ratios. In the process of frequency-dependent selection, female proportion
40 higher or lower than ca. 60% was associated with a reduction or increase in female
41 proportion, respectively, the next year. The rearing of aphid clones in the laboratory
42 indicated that mothers of each clone produced an increasing number of females as
43 maternal size increased. However, the mean male number was not related to maternal
44 size, but varied largely among clones. Given genetic variance in the ability to produce
45 males among clones, selection should favor clones that can produce more numerous
46 males in years with a high female proportion. Population-level sex allocation to females
47 was on average 71%–73% for three localities and more female-biased when maternal
48 conditions were better. This tendency was accounted for by the hypothesis of
49 competition among foundresses rather than the hypothesis of local mate competition.
50 We conclude that despite consistent operation of frequency-dependent selection, the sex
51 ratio continues to fluctuate because environmental conditions always push it away from
52 equilibrium.

53

54 *Keywords:*
55 climate;
56 clonal reproduction;
57 exploitative competition;
58 Eriosomatinae;
59 local mate competition;
60 long-term census;
61 sex allocation;
62 time series.
63

64 **Introduction**

65 Frequency-dependent selection on the proportions of the sexes is a fundamental
66 principle of adaptive sex-ratio evolution, including random mating within a population,
67 local mate competition, and cooperation or competition between relatives (Charnov,
68 1982; Wrensch & Ebbert, 1993; Hardy, 2002; West, 2009). If the ratio of one sex is
69 lower than equilibrium, females that overproduce offspring of the minority sex can
70 propagate their genes more efficiently, and in the next generation the minority sex
71 increases in proportion. Finally, the sex ratios are hypothesized to reach equilibrium,
72 and thereby a stable, balanced sex ratio is realized because of frequency-dependent
73 selection. The hypothesis of frequency-dependent selection was for the first time
74 proposed by Düsing (1884) and explicitly formulated by Fisher (1930). Subsequently,
75 this hypothesis has been widely accepted and incorporated into mathematical models
76 (Frank 1990; Seger & Stubblefield, 2002). In contrast, testing frequency-dependent
77 selection presents several difficulties. The major difficulty is the high stability of
78 population sex ratios, particularly in mammals and birds (Charnov, 1982; Clutton-Brock,
79 1986; Clutton-Brock & Lason, 1986). Exceptions to sex ratio stability include those of
80 fishes and reptiles with environmental sex ratio determination (Charnov & Bull, 1989;
81 Korpelainen, 1990; Janzen & Paukstis, 1991) and arthropods with extremely distorted
82 sex ratios because of infection with *Wolbachia* or other cytoplasmic factors (Werren,
83 1997; Stouthamer *et al.*, 1999; Jiggins *et al.*, 2001; Charlat *et al.*, 2003). Stability of
84 population sex ratios not only makes it difficult to test the operation of
85 frequency-dependent selection, but also only enables us to postulate that random
86 segregation of sex chromosomes in the heterogametic sex is the determinant of 1:1
87 population sex ratios. It is, however, indispensable to know how frequency-dependent
88 selection functions in wild populations to understand the mechanism underlying sex
89 ratio evolution.

90 To date, tests of frequency-dependent selection have been conducted by
91 experimentally perturbing the population sex ratio away from 50% (Conover &
92 Voorhees, 1990; Basolo, 1994; Carvalho *et al.*, 1998; Blows *et al.*, 1999). Initial
93 populations with biased sex ratios were established in these studies by using a

94 temperature-dependent sex determination system in the Atlantic silverside, *Menidia*
95 *menidia*, a single-locus, three-factor system of sex determination in the platyfish,
96 *Xiphophorus maculatus*, a X-Y meiotic drive system in *Drosophila mediopunctata*, and
97 hybridization between two *Drosophila* species. These studies showed that population
98 sex ratio approached 50% males at different rates of evolution ranging from 3 to 330
99 generations, depending on the genetic basis of sex ratio determination, thus confirming
100 frequency-dependent selection. However, few studies have documented the presence of
101 frequency-dependent selection on sex ratios in wild populations. One exceptional
102 instance was a drastic increase in the male proportion of the butterfly *Hypolimnas*
103 *bolina* caused by the rapid spread of a male-rescue allele in populations infected with
104 male-killing *Wolbachia*; that is, in a population with a highly female-biased sex ratio
105 (Charlat *et al.*, 2007). This example suggests that a biological or environmental factor
106 that perturbs the sex ratio away from equilibrium is required to detect
107 frequency-dependent selection in the wild.

108 Eriosomatine aphids have often been used in sex ratio studies because maternal
109 investment in male and female offspring is readily evaluated through dissection of
110 mothers (Yamaguchi, 1985; Kindlmann & Dixon, 1989; Kurosu & Aoki, 1991; Foster &
111 Benton, 1992; Moran, 1993; Foster, 2002; Akimoto & Yamaguchi, 2004; Akimoto &
112 Murakami, 2012; Akimoto *et al.*, 2012). Aphid mothers parthenogenetically produce
113 male eggs by eliminating one of the X chromosomes, and as such, they can control the
114 sex ratio in the brood. In the Eriosomatinae, winged aphids emerging in autumn
115 parthenogenetically produce male and female embryos in their abdomen and give birth
116 to them on the trunk of the primary host. After birth, without taking food, males and
117 females molt and copulate only using resources provided by their mother. Thus, body
118 mass of offspring is completely determined by maternal investment. Females in this
119 generation produce a single egg, which occupies most of her body. Because female
120 embryos are larger than male embryos (Lampel, 1968-69; Yamaguchi, 1985), maternal
121 investment in both sexes should be evaluated as percentage resource allocation to both
122 sexes when the hypothesis of equal allocation is tested. If production costs for male and
123 female offspring can be assessed appropriately, we could precisely quantify maternal

124 resource allocation to the two sexes in the brood, in addition to determining the
125 numerical sex ratios of broods and populations (Akimoto *et al.*, 2012).

126 The pioneer work in the Eriosomatine was undertaken by Yamaguchi (1985), who
127 put forward the constant male hypothesis (Frank, 1987) for *Prociphilus oriens*
128 Mordvilko, 1935 and provided evidence for it by postulating local mate competition
129 (LMC) among males of the same brood. The constant male hypothesis states that given
130 variation in reproductive resources among mothers and LMC, each mother should
131 produce the same number of males and that the remaining resources should be allocated
132 to females (Frank, 1985; Yamaguchi, 1985). Therefore, this hypothesis predicts that
133 mothers with a larger amount of resources should produce more female-biased broods.
134 Later studies revealed exceptions to the constant male hypothesis (Moran, 1993;
135 Akimoto *et al.*, 2012). In eriosomatine species, including *P. oriens*, some fraction of
136 mothers produce all-female broods, which are fertilized by males from other mothers.
137 Nevertheless, Akimoto *et al.* (2012) indicated that there is still a significant tendency for
138 larger *P. oriens* mothers to produce larger broods with a more female-biased sex
139 allocation. This study suggests that *P. oriens* mothers have the potential to adjust sex
140 allocation of their offspring depending on the amount of resources they acquired during
141 development.

142 Given condition-dependent sex allocation in *P. oriens*, population-level sex
143 allocation can be expected to vary among localities and years, as in other animals with
144 an environmental sex determination system (Conover & Heins, 1987; Charnov & Bull,
145 1989; Korpelainen, 1990; Janzen, 1994; Ospina-Álvarez & Piferrer, 2008). In fact, a
146 significant difference in the population sex allocation was detected among different
147 localities (Akimoto *et al.*, 2012). Autumnal winged females (mothers) develop on the
148 roots of the secondary host plant, *Abies sachalinensis* (Fig. 1), and their growth
149 condition depends on the conditions of the host plant. We hypothesized that if climatic
150 conditions affected the growth of host trees, then maternal resources, on average, would
151 increase in years when good climate induces vigorous growth of host plants, and vice
152 versa, resulting in yearly fluctuations in maternal resources and the resulting population
153 sex allocation. Fluctuations in population sex allocation from year to year would

154 provide an opportunity to test frequency-dependent selection in the wild.

155 To understand selective pressures on and the genetic basis of sex allocation
156 control in *P. oriens*, we have established three research goals: (1) long-term censuses for
157 numerical sex ratios, (2) assessment of female fertility under varying sex ratio
158 conditions, and (3) assessment of clonal sex allocation through rearing of clones. Firstly,
159 we recorded the population sex ratios for 14 consecutive years or more at three
160 localities to evaluate the variation among years and localities. We aimed to understand
161 which factors were responsible for the variation and tested if frequency-dependent
162 selection was acting on the numerical sex ratio. Secondly, we examined the factors that
163 were linked to reproductive failure in females in the breeding populations. Thirdly, we
164 attempted to rear *P. oriens* clones to understand how clones control their sex ratios
165 under variable host conditions. Synthesizing this information, we demonstrated how and
166 why the population sex ratio fluctuates widely in *P. oriens*, and why
167 frequency-dependent selection was detected specifically in *P. oriens*.

168 Host-alternating aphids reproduce parthenogenetically from spring to autumn,
169 with different female morphs appearing continuously (Fig. 1). This study focused on
170 autumnal winged females and the following sexual generation. To distinguish the two
171 generations, the sexual female is hereafter referred to as the ‘female’, whereas the
172 autumnal winged female is hereafter referred to as the ‘mother’ or sexupara. Males and
173 sexual females are collectively called ‘sexuals’. Some sexuparae produce bisexual
174 broods in their abdomen (Fig. 1), whereas others produce all-female broods (Akimoto *et*
175 *al.*, 2012), and these were abbreviated as all-F sexuparae.

176 In the following analyses, we used female proportion in a population as an index
177 of the numerical sex ratio. Our previous study showed that the production cost of one
178 female is, on average, 1.85 times as much as that of one male (Akimoto *et al.*, 2012), so
179 that we calculated a mother's investment in the brood as the number of male embryos
180 plus 1.85 times the number of female embryos. Population-level investment in males
181 and females was, respectively, represented by the sum of the numbers of male embryos
182 and 1.85 times the sum of the numbers of female embryos produced by the sexuparae
183 examined. The population-level allocation ratio to females was defined as population

184 investment in females divided by the sum of population investment in males and
185 females, i.e., (1.85 times the total female number) / (1.85 times the total female number
186 + the total male number).

187

188

189 **Materials and methods**

190 **Aphid collection**

191 Autumnal winged females (sexuparae) appear abundantly on calm days in mid and late
192 October, and a large swarm is formed around *Fraxinus mandshurica* trees, the primary
193 host. Sexuparae were collected around *F. mandshurica* trees during flying using an
194 insect net (Akimoto *et al.*, 2012). Aphid species usually include obligatorily
195 parthenogenetic lineages in the population (Simon *et al.*, 2002). However, this species
196 consists only of cyclical parthenogenetic lineages.

197 Sexuparae were collected in three sites in Hokkaido, northern Japan: (1) the
198 campus of Hokkaido University, Sapporo (43° 4' 12" N, 141° 20' 22" E), (2)
199 Kasuga Cho, Iwamizawa (43° 11' 30" N, 141° 46' 48" E), and (3) Forestry
200 Research Institute, Hokkaido Research Organization, Bibai (43° 17' 3" N, 141° 51'
201 11" E), where Yamaguchi's study (1985) was conducted. The distance between Sapporo
202 and Iwamizawa was 38.3 km, that between Sapporo and Bibai was 47.6 km, and that
203 between Iwamizawa and Bibai was 11.4 km. Collection was yearly conducted from
204 2002 to 2015 in all the three localities, and these samples were used for comparison of
205 sex ratios among the localities. In addition, samples collected in Sapporo in 1994 and
206 2000, samples collected in Iwamizawa in 1998, 2000 and 2001, and samples collected
207 in Bibai from 1981 to 2000 (data of some years are missing) were added to some
208 analyses.

209 Sexuparae collected on one day of mass flying were used for representing the sex
210 ratio in the locality because a previous study showed no significant differences in sex
211 ratios among collection days in the period of mass flying (Akimoto *et al.*, 2012).
212 Collected sexuparae were preserved in vials of 80% ethanol and then dissected in a Petri
213 dish under a binocular to count the numbers of male and female embryos. In each of

214 population samples, on average, 167 (\pm 67.8 (SD)) sexuparae were randomly selected,
215 and their abdomen was dissected in 80% ethanol by using two fine insect pins to take
216 out embryos. Embryos of each sex were of almost the same size, and female embryos
217 were larger in size with a wider abdomen. Embryos were readily sexed under a
218 binocular on the basis of their size and shape.

219 Variance among mothers' investments was decomposed into three variance
220 components; the component among years, that among localities within years, and that
221 within localities, and the percentage contributions of these components were calculated
222 based on the restricted maximum likelihood method (REML) using JMP ver.9.0.2. (SAS
223 Institute Inc., Cary, NC, USA).

224

225 **Oviposition success in different sex ratios**

226 To explore the relationship between the sex ratio in a breeding population and female
227 oviposition success, we transferred different numbers of virgin males and females in an
228 experimental arena and examined how many females successfully oviposited their
229 single eggs. For this experiment, eight treatments were prepared; 1) 12 males + 2
230 females (with 52 replicates), 2) 12 males + 4 females (with 25 replicates), 3) 12 males +
231 6 females (with 8 replicates), 4) 12 males + 12 females (with 25 replicates), 5) 4 males
232 + 12 females (with 25 replicates), 6) 1 male + 12 females (with 25 replicates), 7) 1 male
233 + 43 females (with 14 replicates), and 8) 1 male + 50 females (with 14 replicates). All
234 experiments except 7) were conducted in 2013 and the remaining experiment was
235 prepared in 2015. Immature males and females were collected from the Iwamizawa
236 population. At the evening of the day of sexupara mass flying, paper gauze (Haize
237 gauze, Asahikasei[®]) that had been placed on the branches of *F. mandshurica* was
238 collected and brought to the laboratory. Newly born sexuals were collected from the
239 paper gauze. At 16°C, 44 h and 51 h, respectively, were needed for males and females to
240 reach sexual maturation. We transferred males and females to the arena before their
241 final molt. The experiments were conducted at 16°C using small styrol containers (30 ×
242 30 × 10 mm) lined with a sheet of dampened filter paper.

243

244 **Clonal sex ratios**

245 In order to understand clonal sex ratios, we reared *P. oriens* colonies clonally on the
246 twigs of the primary host, *Fraxinus mandshurica* and subsequently on the roots of the
247 secondary host, *Abies sachalinensis*, until the appearance of sexuparae (see Fig. 1).
248 *Fraxinus mandshurica* twigs, ca. 50 cm long, were cut off in early May and
249 hydroponically cultured in plastic bottles (750ml) filled with water at room
250 temperatures in the laboratory. Hatched first instar foundresses, collected from the
251 Iwamizawa population, were transferred onto the cut twigs by using a fine writing brush,
252 and they were allowed to grow to adulthood and reproduce parthenogenetically. To
253 prevent contamination of different clones, we maintained a single foundress that
254 reproduced earliest on the twig by removing others. Foundresses produced about 50
255 second-generation larvae, which moved to growing leaves and developed into winged
256 adults in late May in the laboratory. Winged adults from the same clone were collected
257 into small styrol containers (30 × 30 × 10 mm), which were lined with a sheet of
258 dampened filter paper. As a stimulant of larviposition, we placed a young shoot of *Abies*
259 *sachalinensis* in the container. Thirty six to 48 h later, winged adults
260 parthenogenetically produced first instars of the third generation, which were used for
261 the establishment of the clonal colony on the roots of a single *A. sachalinensis* tree.

262 Prior to the transfer of first instars, young *A. sachalinensis* trees ca. 60 cm tall
263 were transplanted in pots in early May, and they were maintained in a garden of private
264 land in Iwamizawa, Hokkaido (43° 11' N, 141° 46' E). In 2008, 2009 and 2011, we
265 prepared two, 12, and 12 potted trees, respectively. Because *Prociphilus oriens* colonies
266 maintain mutualistic relationships with *Lasius* ants on *A. sachalinensis* roots from early
267 June to mid October, rearing of *P. oriens* colonies requires continuous visits and
268 attendance by the ants. To keep constant visits of *Lasius japonicus* workers, we put
269 diluted honey on the basal stems of *A. sachalinensis* trees twice a day using a dropper.
270 After workers were always observed to forage around the stem bases, first instars that
271 were born in the containers were released to the stem base using forceps. First instars
272 from different clones were separately transferred onto different *A. sachalinensis* trees.
273 However, in 2011 first instars of one clone were transferred onto two trees to understand

274 the effect of host trees on the sex allocation. We observed that *Lasius japonicus* workers
275 soon approached and tapped first instars with their antennae, and carried them into an
276 ant nest near the stem base with holding them between their mandibles. The release of
277 first instars was conducted each time when they were born in the container. After the
278 release, each potted *A. sachalinensis* tree was covered with a net to prevent other
279 winged adults from alighting on the tree until the appearance of winged adults ended.
280 These trees were kept intact during summer, and again netted from late September on to
281 collect sexuparae emerging from the trees. Sexuparae were daily collected into vials of
282 80% ethanol. Finally, *P. oriens* colonies on two (100%), seven (58.3%), and seven
283 (58.3%) *A. sachalinensis* trees successfully produced sexuparae in 2008, 2009 and 2011,
284 respectively.

285 After the collection of sexuparae, the total number was counted for each *A.*
286 *sachalinensis* tree (colony). Fifty to 68 sexuparae were selected per colony, and each
287 adult was dissected in a droplet of Hoyer's medium on a slide glass by using two fine
288 insect pins. For each sexupara, male and female embryos and one wing were mounted
289 under two cover glasses on a single slide glass. The mounted specimens were then
290 placed at 60°C in an incubator for 3 h. Wing length was measured by using ImageJ
291 version 1.47 (Abràmoff *et al.*, 2004, available from <http://rsbweb.nih.gov/ij/>), and the
292 relationship between maternal wing length and the numbers of male and female
293 embryos was examined. We also evaluated the numerical sex ratio per mother and a
294 mother's total investment as mentioned above.

295

296 **Statistics**

297 The basic statistics for the numbers of males and females produced by a mother were
298 calculated for the Bibai population, in which samples were collected for the longest
299 term.

300 To test if the observed yearly fluctuations in female proportions tend to return to a
301 specific value (i.e., they show stationarity) or not, we first conducted the unit root test
302 for the time-series data in the three localities using the Augmented Dickey-Fuller test
303 (adf.test) in R version 3.2.1 (R Core Team, 2015). If stationarity is rejected by the test

304 (i.e., fluctuations follow a random walk process), then the data will be composed of
305 dependent samples, possibly producing a seeming trend with time. This result violates
306 the assumption of correlation and regression analyses. Thus, in comparisons between
307 different time-series data, we took difference between neighboring years before we
308 calculate the correlation between two time-series data.

309 We tested if fluctuations in the proportions of females and all-F sexuparae and
310 maternal investment are correlated with environmental variables or not by using
311 stepwise multiple regression analysis. In the model, localities (three sites) and six
312 metrological parameters during the growing season (precipitation in July, August and
313 September, and mean temperature in July, August and September) at each locality were
314 specified as explanatory variables. The explanatory variables were selected in a
315 stepwise manner (the backward method) based on the Bayesian information criterion
316 (BIC) using JMP ver.9.0.2. Metrological data at three localities (Bibai, Iwamizawa, and
317 Sapporo) were obtained from the database of Japan Metrological Agency
318 (<http://www.data.jma.go.jp/obd/stats/etrn/index.php>). In addition, we tested to what
319 extent female proportion in a year affected changes in that proportion the next year
320 (proportion in year i – proportion in year $i-1$) by using ANCOVA. In the model, change
321 in female proportion in year i was treated as the response variable, while female
322 proportion in year $i-1$, locality, and the interaction between them were treated as
323 explanatory variables.

324 In mating experiments, the number of females that successfully produced
325 fertilized eggs relative to the number of females that failed was analyzed using logistic
326 regression, in which female proportion in experimental arenas and its square were
327 treated as explanatory variables. The square term was added to the model because
328 preliminary observations showed that female proportions in arenas had non-linear
329 effects on female fertility. The glm function in R (R Core Team, 2015) was used for the
330 analysis with a binomial error structure.

331 In rearing experiments, factors affecting the occurrence of all-F sexuparae were
332 tested with logistic regression. In the model, clones and maternal wing length were
333 specified as explanatory variables, and whether a brood is bisexual or includes females

334 only was specified as the binary response variable. Subsequently, we evaluated factors
335 affecting the number of females a mother produced using ANCOVA, in which clones,
336 maternal wing length, and the interaction between them were treated as explanatory
337 variables. The same analysis was also applied to the number of males per mother.

338 To know whether the numerical sex ratio per mother varies significantly among
339 colonies or not, we analyzed the numbers of female embryos relative to male embryos
340 by using the glm function with a binomial error structure. Difference in the sex ratio
341 between any two of the 16 colonies (120 pairwise combinations) was tested, and the
342 sequential Bonferroni adjustment (Rice, 1989) was applied to the calculated *P* values to
343 keep the table-wide *P* value constant at 0.05.

344

345 **Results**

346 **Time series analysis**

347 During the period from 1981 to 2015, sexuparae in the Bibai population produced zero
348 to four male embryos (mean = 2.89 ± 1.56 (SD)) and a variable number of female
349 embryos ranging from zero to more than 11 (mean = 4.16 ± 2.57 ; Table 1).
350 Approximately 60% of sexuparae produced four males, but 17% produced all females.
351 The proportion of sexuparae producing four males and that of sexuparae producing all
352 females in each year were negatively correlated throughout the period (Spearman's $r =$
353 -0.865 , $n = 20$, $P < 0.0001$), and thus we focused only on the proportion of all-F
354 sexuparae in later analyses.

355 Fluctuations in female proportions were shown for the Sapporo, Iwamizawa, and
356 Bibai populations (Fig. 2), along with fluctuations in the proportions of all-F sexuparae.
357 Female proportions fluctuated widely from 46.5% to 67.8% (mean = $57.2\% \pm 6.0$) in
358 Sapporo, from 49.1% to 76.0% (mean = $59.5\% \pm 8.5$) in Iwamizawa, and from 46.4% to
359 84.3% (mean = $59.4\% \pm 11.0$ (SD)) in Bibai. In initial study years (in 1981 (data not
360 shown) and 1993) in Bibai, the proportion of all-F sexuparae was less than 3% but
361 gradually increased and started to fluctuate widely after 2002, with a maximum of
362 52.3% in 2009. Population-level resource allocation was highly female-biased;
363 allocation to females was, on average, 72.3% throughout the populations (71.3% in

364 Sapporo, 73.1% in Iwamizawa, and 72.5% in Bibai).

365 Augmented Dickey-Fuller tests indicated that the hypothesis of random walk was
366 not rejected for the 2002–2015 data of the Iwamizawa and Bibai populations,
367 suggesting that the time-series of female proportions are not stationary (for Iwamizawa,
368 Dickey-Fuller = -1.96, $P = 0.587$; for Bibai, Dickey-Fuller = -1.70, $P = 0.687$). In
369 contrast, stationarity was confirmed for the Sapporo population (Dickey-Fuller = -4.07,
370 $P = 0.021$). This result implies that stabilizing forces act more intensely on fluctuations
371 in the Sapporo population. However, since fluctuations in the Iwamizawa and Bibai
372 populations are non-stationary, correlation analyses involving these fluctuations do not
373 satisfy the condition of independence of data. Thus, the following correlation analyses
374 were performed by taking differences between neighboring years.

375 The fluctuations of female proportions in the Bibai population were significantly
376 correlated with those in the Sapporo and Iwamizawa populations (for Sapporo, $n = 13$, r
377 = 0.561, $P = 0.0462$; for Iwamizawa, $n = 13$, $r = 0.620$, $P = 0.0239$). However, no
378 significant correlation was detected between the Sapporo and Iwamizawa populations (n
379 = 13, $r = 0.166$, $P = 0.587$). Fluctuations in the proportions of all-F sexuparae were
380 significantly correlated between all combinations of the Sapporo, Iwamizawa, and Bibai
381 populations (for Sapporo-Iwamizawa, $n = 13$, $r = 0.641$, $P = 0.0182$; for Sapporo-Bibai,
382 $n = 13$, $r = 0.675$, $P = 0.0114$; for Iwamizawa-Bibai, $n = 13$, $r = 0.784$, $P = 0.0015$). This
383 result implies effects of regional factors common to the three localities on these
384 fluctuations. In every population, fluctuations in female proportions were highly and
385 positively correlated with those in all-F sexupara proportions (Fig. 2; for Sapporo, $n =$
386 13, $r = 0.850$, $P = 0.0002$; for Iwamizawa, $n = 15$, $r = 0.879$, $P < 0.0001$; for Bibai, $n =$
387 13, $r = 0.947$, $P < 0.0001$). Similarly, fluctuations in female proportions were positively
388 correlated with those in the mean maternal investment (for Sapporo, $n = 13$, $r = 0.917$, P
389 < 0.0001 ; for Iwamizawa, $n = 15$, $r = 0.854$, $P < 0.0001$; for Bibai, $n = 13$, $r = 0.787$, P
390 < 0.0014). Of the variance among mothers' investments, 5.9% was accounted for by the
391 among-year component, 8.0% was by the among-locality component, and 86.1% was by
392 the within-locality component.

393 Stepwise multiple regression analysis indicated that climatic factors during the

394 growing season were highly correlated with the proportions of females and all-F
395 sexuparae and mean maternal investment; these response variables decreased with
396 increasing precipitation in August and increasing temperature in September, while the
397 mean maternal investment increased with increasing temperature in August (Table 2).

398 Female proportion in a year had a significantly negative effect on changes in that
399 proportion the next year (ANCOVA, $R^2 = 0.55$; $df = 1,35$, $F = 36.65$, $P < 0.0001$; Fig.
400 3); a value higher than ca. 60% in a year led to a large reduction in female proportion
401 the next year, whereas a value $<$ ca. 60% resulted in an increase in female proportion the
402 next year. However, neither of locality and the interaction between female proportion
403 and locality affected changes in female proportion in the next year (for locality, $df =$
404 $2,35$, $F = 1.13$, $P = 0.334$; for the interaction, $df = 2,35$, $F = 1.52$, $P = 0.232$).

405 The previous analysis indicated the significant effect of climatic factors on female
406 proportion. Thus, we recalculated the effect of frequency-dependent selection by adding
407 precipitation in August and temperature in September to the model (Table 3). Analysis
408 based on this model (ANCOVA, $R^2 = 0.70$) resulted in lower AIC (288.0) than that in
409 the previous model (302.6) and revealed significant effects of precipitation in August
410 and female proportion in the previous year (Table 3).

411

412 **Oviposition success in different sex ratios**

413 The proportion of females that successfully produced fertilized eggs exhibited a
414 unimodal pattern to female proportion (Fig. 4). The oviposition success rate was
415 positively correlated with female proportion ($z = 8.15$, $P < 0.0001$) and negatively with
416 the square of female proportion ($z = -9.61$, $P < 0.0001$); the oviposition success rate
417 reached a maximum when male number was one third of female number (Fig. 4). In
418 arenas where more than 40 females were present per male ($n = 28$), males fertilized a
419 median of 18 females and a maximum of 35 females. In arenas where males are more
420 numerous than females, females often died during molting or oviposition due to
421 interference from males.

422

423 **Clonal sex ratios**

424 From 2008 to 2011, we successfully reared 15 aphid clones for which the relationships
425 among maternal size, maternal investment and female proportions were evaluated. All
426 clones but one produced all-F sexuparae, although their proportion varied largely
427 among clones (Fig. 5), ranging from 79.0% in clone C to 0 % in clone LY5. This result
428 suggests that all-F sexuparae did not come from specific clones. The proportion of all-F
429 sexuparae in a clone was highly correlated with female proportion among the clones (r
430 = 0.882, $n = 15$, $P < 0.0001$). In 2008 and 2009, most clones produced all-F sexuparae
431 at the highest proportion, but in 2011 four of six clones produced sexuparae producing a
432 4-male brood at the highest proportion (Fig. 5). This tendency was similar to the
433 patterns of male numbers in the Iwamizawa population, from which the clones were
434 derived; the proportion of sexuparae producing a 4-male brood increased abruptly in
435 2011 (Fig. 5). Logistic regression showed that the proportion of all-F sexuparae varied
436 significantly among clones ($df = 14$, $\chi^2 = 233.9$, $P < 0.0001$), but was not correlated with
437 maternal wing length ($df = 1$, $\chi^2 = 0.60$, $P = 0.437$).

438 The total number of sexuparae emerging from each colony was not correlated
439 with the clonal sex ratio (Table 4, $r = -0.060$, $n = 15$, $P = 0.832$) or the mean wing
440 length of sexuparae ($r = -0.152$, $n = 15$, $P = 0.589$). Among clones, maternal investment
441 averaged over clone members was positively correlated with the averaged wing length
442 of mothers ($r = 0.864$, $n = 15$, $P < 0.0001$). ANCOVA indicated that female number per
443 mother varied significantly among clones ($df = 14,752$, $F = 21.9$, $P < 0.0001$) and was
444 correlated with maternal wing length ($df = 1,752$, $F = 447.4$, $P < 0.0001$); the
445 interaction between clones and maternal wing length also had a significant effect on
446 female number ($df = 14,752$, $F = 6.0$, $P < 0.0001$). In addition, the mean female number
447 per mother was significantly correlated with the mean maternal investment ($r = 0.630$, n
448 = 15, $P < 0.0001$). Male number per mother varied significantly among clones ($df =$
449 14,752, $F = 26.0$, $P < 0.0001$), but was not correlated with maternal wing length ($df =$
450 1,752, $F = 0.65$, $P = 0.420$) or affected by the interaction between them ($df = 14,752$, F
451 = 1.44, $P = 0.129$).

452 The numerical sex ratio was compared among 16 colonies on *A. sachalinensis*
453 trees, two of which (LY5 and LY6) were derived from the same clone (Table 4). No

454 significant difference was found in sex ratio between LY5 and LY6 colonies.

455

456 **Discussion**

457 Because of the widely held belief that the sex ratio is balanced and maintained at
458 equilibrium, long-term studies on sex ratio variation have rarely been conducted. Our
459 long-term censuses on the sex ratio of *P. oriens* revealed that female proportion
460 fluctuated widely from year to year. It should be noted that female proportion in a year
461 affected the proportion the next year in all of the three populations; when female
462 proportion is higher or lower than ca. 60% in a year, it tended to decrease or increase,
463 respectively, the next year. Therefore, this analysis shows that frequency-dependent
464 selection has been acting on the sex ratio. This study is the first report of
465 frequency-dependent selection acting over a long period in the wild. The present study
466 also indicates that the amount of maternal investment, which is highly correlated with
467 maternal size, fluctuated from year to year. Among-year variation in maternal
468 investment accounted for 5.9 % of the entire variance. We observed higher female
469 proportions in years when mothers had a larger amount of reproductive investment on
470 average. These findings imply that the growth conditions of host plants probably
471 influenced the reproductive investment of mothers, who could adjust sex allocation
472 according to the resources they acquired during development.

473 The finding that fluctuation patterns of female proportion and all-F sexupara
474 proportion were similar among the three localities suggests that these proportions are
475 influenced by large-scale regional factors such as climate. A statistical model
476 incorporating metrological variables verified this hypothesis; high precipitation in
477 August and high temperature in September were strongly linked to decreases in mean
478 maternal investment, female proportion, and all-F sexupara proportion. In Hokkaido,
479 higher precipitation in August typically comes from heavy rainfall caused by typhoons.
480 We speculate that heavy rain may have hindered the aphids' feeding activities on the
481 host roots and detrimentally affected maternal resources. In addition, high temperature
482 in September may have negatively affected the physiological conditions of *Abies*
483 *sachalinensis*, which is adapted to cold climates. In contrast, high temperature in August

484 is linked to longer sunshine duration, which may have promoted the photosynthesis and
485 growth of host trees and resultantly led to a larger amount of maternal investment. In
486 aphids, environmental impacts on a generation are reported to influence the phenotypes
487 of later generations through the telescoping of generations (Mondor *et al.*, 2004;
488 Podjasek *et al.*, 2005; Keiser & Mondor, 2013; Tegelaar *et al.*, 2013). Thus, climatic
489 impacts could affect the population sex ratio through transgenerational effects.

490 Frequency-dependent selection should have acted as the stabilizing force for
491 fluctuations in female proportion, leading to an equilibrium of ca. 60%. However, there
492 was no evidence that fluctuations in female proportions are attenuating with time. We
493 detected large residual variance in the regression analysis of changes in female
494 proportion (Fig. 3), and 38% of the residual variance was attributed to yearly changes in
495 climatic factors. The results of the ANCOVA indicated that both frequency-dependent
496 selection and climatic factors accounted for the fluctuations of female proportions. This
497 result suggests that climatic changes caused wide fluctuations in female proportions,
498 which were in turn adjusted by frequency-dependent selection. Therefore, in *P. oriens*
499 whose sex allocation is affected environmentally, fluctuations in female proportions
500 would not decline despite consistent operation of frequency-dependent selection.

501 The arena experiments with different sex ratios revealed that males enjoyed
502 reproductive advantages when they were minority. Males have the ability to inseminate
503 as many as 18 females, and this high ability may be an adaptation to high female
504 proportions. In this situation, natural selection would act positively on clones that can
505 produce more males. Male proportion was approximately 40% on average in the wild,
506 implying that male-male competition should be moderate. However, when male
507 proportion became higher than 50%, females less successfully produced fertilized eggs
508 because of persistent male courtship behavior, which often disturbed female molting
509 and oviposition. In 17% of 53 population samples, male proportion exceeded 50%. With
510 increasing proportion of males, clones producing higher proportion of females would be
511 favored by selection to mitigate interference from males and male-male competition.
512 Therefore, the mating experiment suggests that females adapt to breeding populations
513 with a high female proportion (ca. 75%), where they can produce fertilized eggs most

514 safely.

515 The fact that sex ratio is condition-dependent has been reported in some aphid
516 species (Yamaguchi, 1985; Foster & Benton, 1992; Moran, 1993; Miller & Aviles,
517 2000; Wool & Sulami, 2001; Foster, 2002; Dagg & Vidal, 2004; Akimoto & Murakami,
518 2012; Akimoto *et al.*, 2012), and this characteristic has almost always been explained in
519 terms of LMC. The present study showed that population-level allocation to females
520 was 71%–73% for the three localities, implying that strong LMC might have occurred
521 in the breeding populations. However, whether the LMC hypothesis is the only rationale
522 for female-biased sex allocation in aphids was questioned by Akimoto *et al.* (2012),
523 who pointed out that female-biased and condition-dependent sex allocation could have
524 evolved from aphid life cycles.

525 Aphid males appear only in one generation and mate with sexual females to
526 produce fertilized eggs. Only females hatch from fertilized eggs, founding new clonal
527 lineages (so called foundresses). In Eriosomatine aphids, body mass of a first-instar
528 foundress is solely determined by the sexupara's investment in each female because
529 every female, without feeding, deposits one large egg, from which a first-instar
530 foundress hatches (Heie, 1980; Fig. 1). Thus, maternal investment in females is
531 equivalent to investment in her offspring foundresses (granddaughters). In aphids, a
532 sexupara's granddaughters (foundresses) and their clonal offspring could compete with
533 unrelated females over limited resources on the same host plant. In this situation, if a
534 mother's extra investment in females can positively contribute to the outcome of
535 competition among foundresses, then the mother could gain higher marginal genetic
536 returns from investment in females than in males.

537 Several sex allocation theories indicate that if the function that relates maternal
538 investment with genetic returns is different between the two sexes, then equal allocation
539 is not expected (Trivers & Willard, 1973; Charnov, 1979; Maynard Smith, 1980; Frank,
540 1990; Seger & Stubblefield, 2002). Theory predicts that if a mother's fitness returns
541 from investment in sons (x) and daughters ($1 - x$) are respectively represented as x^a and
542 $(1 - x)^b$, then the ESS sex allocation to sons (x') is calculated as $x' = a / (a + b)$ (Seger &
543 Stubblefield, 2002). Therefore, in case of $b > a$, that is, if investment in females results

544 in higher marginal returns, the sex ratio is expected to be female-biased. In this
545 theoretical framework, Trivers & Willard (1973) predicted that if mothers have different
546 amounts of reproductive resources, mothers should exhibit different sex allocation
547 patterns, with more fecund mothers allocating more toward the sex with the greater rate
548 of reproductive returns. These non-linear models (Frank, 1990) can be applied to aphid
549 life cycles, in which competition could occur among foundresses. Given competition
550 among unrelated foundresses, a mother's sex allocation would be distorted by selection
551 so as to enhance the reproductive output of her offspring foundresses. In contrast,
552 obvious fighting has not been observed among males in this study or by Foster &
553 Benton (1992). Therefore, more intensive competition between unrelated foundresses
554 than male-male competition may have led to the evolution of female-biased sex
555 allocation. This prediction can be applied to other organisms with cyclic
556 parthenogenesis if the breeding population consists mainly of unrelated individuals.

557 Competition in the foundress generation can arise in two ways, physical fighting
558 and exploitative competition. Examples of combat are known from gall-forming aphids,
559 in which first-instar foundresses fight with one another over the ownership of a suitable
560 galling site or an incipient gall (Whitham, 1979; Aoki & Makino, 1982; Akimoto, 1988;
561 Akimoto & Yamaguchi, 1997). During fighting, larger body size is overwhelmingly
562 advantageous (Whitham, 1979; Aoki & Makino, 1982; Akimoto & Yamaguchi, 1997).
563 In *Tetraneura sorini*, foundresses are reportedly subject to directional selection for
564 larger body size in all populations examined (Muramatsu & Akimoto, 2016). Therefore,
565 where unrelated foundresses frequently fight, mothers can gain higher fitness returns by
566 investing more resources in individual females, thereby producing larger foundresses. In
567 *T. sorini*, highly female-biased sex allocation has been reported (Akimoto & Yamaguchi,
568 2004).

569 In species whose foundresses do not fight, including *P. oriens*, investment of
570 more resources in females is also advantageous for mothers. If a mother can produce
571 more females than do others, she can have more granddaughters (foundresses) in spring,
572 thereby acquiring a head-start advantage over the succeeding exploitative competition
573 among clones. If a larger number of offspring foundresses can occupy more new leaves,

574 then they will overwhelm unrelated foundresses more easily on the same plant. In
575 addition, Akimoto (2006) indicated that in *P. oriens*, greater investment in a female (and
576 her egg) resulted in the foundress with disproportionately higher fecundity. This is
577 because the allometry of foundress gonad mass to egg size is positive with an allometry
578 coefficient of 1.5. In aphids, exploitative competition among conspecifics or
579 heterospecific clones is reportedly severe (Moran & Whitham, 1990; Inbar *et al.*, 1995;
580 Fuller *et al.*, 1999). Furthermore, the head-start effects have been corroborated by the
581 prevalence of asexual clones in areas where sexual and asexual lineages coexist (Rispe
582 & Pierre, 1998; Rispe *et al.*, 1998; Dedryver *et al.*, 2001). When sexual clones hatch
583 from overwintered eggs in spring, asexual clones have already begun to reproduce on
584 the same plant during mild winters, occupying suitable feeding sites. Thus, in spite of
585 the advantage of genetic diversity, sexual clones are overwhelmed by asexual clones
586 during mild winters.

587 The foundress competition hypothesis for female-biased sex ratios can be applied
588 to almost all aphid species and predicts that more fecund mothers should produce a
589 more female-biased sex ratio (Trivers & Willard, 1973). This tendency has been
590 detected in aphid species whose life cycles are unlikely to lead to LMC (Ward &
591 Wellings, 1994; Miller & Aviles, 2000). In addition to the possibility of foundress
592 competition, local mate competition among sibling males or local resource competition
593 among sibling foundresses (Dagg & Vidal, 2004) might also arise in *P. oriens*. The
594 relative magnitude of the three kinds of competition will determine sex allocation.
595 However, in host-alternating aphids, because a large number (usually thousands) of
596 winged mothers aggregate on a single primary-host tree, we postulate that competition
597 among unrelated foundresses should be much stronger than competition among sibling
598 foundresses or among sibling males. When mothers' density is low and the mobility of
599 males and foundresses is limited, local mate competition and local resource competition
600 will play an important role. However, it should be noted that effects of these two factors
601 are likely to be canceled out in aphids with limited mobility (West, 2009; Akimoto &
602 Murakami, 2012).

603 The appearance of all-F sexuparae cannot be accounted for by the LMC

604 hypothesis (Yamaguchi, 1985) or its modified version (Stubblefield & Seger, 1990),
605 which predicts that mothers produce a fixed number or an increasing number of males
606 as maternal size increases. The LMC hypothesis predicts the optimal sex allocation for
607 mothers joining a local breeding population. However, in aphids, we have to determine
608 the optimal sex allocation strategy of clones rather than individual mothers because the
609 clone is a unit of natural selection (Moran, 1993; Akimoto & Murakami, 2012). The
610 main finding of the rearing experiments was that almost all clones produced two types
611 of sexuparae: those producing an all-female brood and those producing a bisexual brood
612 (mainly including 4 males). We found that *P. oriens* clones exhibited a wide variety of
613 sex ratio expression, with clones producing a male-biased sex ratio, a female-biased sex
614 ratio, or an intermediate ratio (Table 4, Fig. 5). A large variation in sex ratio was
615 detected among clones or mothers in other aphids (*Masonaphis maxima*, Gilbert &
616 Raworth, 1998; *Rhopalosiphum padi*, Rispe *et al.*, 1999; *Tamalia coweni*, Miller &
617 Aviles, 2000; Adelgidae, Steffan, 1970; Sano & Ozaki, 2011). We hypothesize that all-F
618 sexuparae has evolved from sexuparae producing a bisexual brood because of
619 advantages in clone competition; namely, the former type can produce more females
620 efficiently. In the eriosomatine *Kaltenbachiella japonica*, almost all clones produce only
621 sexuparae with bisexual broods, whereas one clone was observed to produce only all-F
622 sexuparae (Akimoto & Murakami, 2012). Dimorphism in *P. oriens* sexuparae may have
623 evolved from the ancestral situation found in *K. japonica*.

624 Analysis of sex-ratio fluctuations indicated that favorable environmental
625 conditions led to increases in maternal investment and all-F sexupara proportion, which
626 in turn increased female proportion. This finding implies that when climatic conditions
627 are favorable, each clone increases the proportion of all-F sexuparae relative to
628 sexuparae producing bisexual broods. The developmental mechanism that determines
629 the two types of sexuparae in a clone remains unknown. The rearing experiment showed
630 that sexupara body size was not the determinant of the dimorphism in each clone. Apart
631 from this environmental effect on the production of all-F sexuparae, individual mothers
632 in every clone had the ability to increase female numbers in response to increasing
633 resources. This condition-dependent female production is probably another mechanism

634 for clones to be advantageous in clonal competition. Therefore, in *P. oriens*, there are
635 two mechanisms (clonal and individual) to increase female number, and as such, female
636 proportion is likely to increase rapidly under favorable environments.

637 The fact that male number per mother was not affected by maternal resources but
638 varied largely among clones suggests that male production is less affected by
639 environmental conditions. In the experiment in which the members of the same clone
640 were reared on different host trees (LY5 and LY6), we found that the numerical sex
641 ratios of the two colonies were highly male-biased and did not vary significantly
642 between the colonies. Although detailed genetic experiments were not conducted, there
643 may be a genetic basis for the male-producing ability of clones. Given genetic variation
644 in male-producing ability, frequency-dependent selection can act on this variation.

645 In conclusion, the present study demonstrated the operation of
646 frequency-dependent selection and a large fluctuation in the sex ratio under the
647 operation of frequency-dependent selection. *Prociphilus oriens* clones can produce a
648 more female-biased sex ratio when they grow in good host conditions, and thus, the
649 population sex ratio fluctuates in response to yearly environmental variation. For
650 organisms without the environmental sex-ratio determination system, it may be difficult
651 to detect frequency-dependent selection because of stable sex ratios. We conclude that
652 although frequency-dependent selection acts consistently, sex ratios continue to
653 fluctuate because environmental conditions drive the sex ratio away from equilibrium.

654

655 **Acknowledgments**

656 We are grateful to Y. Yamaguchi for discussion and kindly providing us a part of her
657 own data. We also thank a number of graduate students of the laboratory of Systematic
658 Entomology, Y. Tanaka, R. Tomisawa, A. Ito, T. Yoshino, R. Mitsuhashi, Y. Matsumura
659 and Y. Ueno for helping in data collection. This research was supported by
660 Grants-in-Aid (number 17370028, 23370037) for Scientific Research from the Japan
661 Society for the Promotion of Science given to S. Akimoto.

662

663 **References**

664 Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. 2004. Image processing with ImageJ.

665 *Biophotonics Int.* **11**: 36–42.

666 Akimoto, S. 1988. The evolution of gall parasitism accompanied by a host shift in the

667 gall aphid, *Eriosoma yangi* (Homoptera, Aphidoidea). *Biol. J. Linn. Soc.* **35**:

668 297–312.

669 Akimoto, S. 2006. Inbreeding depression, increased phenotypic variance, and a

670 trade-off between gonads and appendages in selfed progeny of the aphid

671 *Prociphilus oriens*. *Evolution* **60**: 77–86.

672 Akimoto, S. & Murakami, T. 2012. Condition-dependent sex allocation by clones of a

673 galling aphid. *Behav. Ecol. Sociobiol.* **66**: 1475–1484.

674 Akimoto, S. & Yamaguchi, Y. 1997. Gall usurpation by the gall-forming aphid,

675 *Tetraneura sorini* (Insecta Homoptera). *Ethol. Ecol. Evol.* **9**: 159–168.

676 Akimoto, S. & Yamaguchi, Y. 2004. Evolution of sex allocation in the genus *Tetraneura*

677 (Aphididae: Pemphiginae). In: *Aphids in a New Millennium. Proceedings of the*

678 *Vith International Symposium on Aphids* (J.C. Simon, C.A. Dedryver, C. Rispe

679 & M. Hulle, eds), pp. 201–206. INRA Editions, Versailles.

680 Akimoto, S, Mitsuhashi, R. & Yoshino, T. 2012. Female-biased sex allocation in wild

681 populations of the eriosomatine aphid *Prociphilus oriens*: local mate competition

682 or transgenerational effects of maternal investment? *Popul. Ecol.* **54**: 411–419.

683 Aoki, S. & Makino, S. 1982. Gall usurpation and lethal fighting among fundatrices of

684 the aphid *Epipemphigus niisimae* (Homoptera, Pemphigidae). *Kontyu* **50**:

- 685 365–376.
- 686 Basolo, A.L. 1994. The dynamics of Fisherian sex ratio evolution: theoretical and
687 experimental investigations. *Am. Nat.* **144**: 473–490.
- 688 Blows, M.W., Berrigan, D. & Gilchrist, G.W. 1999. Rapid evolution toward equal sex
689 ratios in a system with heterogamety. *Evol. Ecol. Res.* **1**: 277–283.
- 690 Carvalho, A.B., Sampaio, M.C., Varandas, F.R. & Klackzo, L.B. 1998. An experimental
691 demonstration of Fisher’s principle: evolution of sexual proportions by natural
692 selection. *Genetics* **148**: 719–731.
- 693 Charlat, S., Hurst, G.D.D. & Merçot, H. 2003. Evolutionary consequences of *Wolbachia*
694 infections. *Trends Genet.* **19**: 217-223.
- 695 Charlat, S., Hornett, E.A., Fullard, J.H., Davies, N., Roderick, G.K., Wedell, N. & Hurst,
696 G.D.D. 2007. Extraordinary flux in sex ratio. *Science* **317**: 214.
- 697 Charnov, E.L. 1979. Simultaneous hermaphroditism and sexual selection. *Proc. Natl.*
698 *Acad. Sci. USA* **76**: 2480-2484.
- 699 Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press,
700 Princeton.
- 701 Charnov, E.L. & Bull, J.J. 1989. Non-fisherian sex ratios with sex change and
702 environmental sex determination. *Nature* **338**: 148–150.
- 703 Clutton-Brock, T.H. 1986. Sex ratio variation in birds. *Ibis* **128**: 317-329.
- 704 Clutton-Brock, T.H. & Lason, G.R. 1986. Sex ratio variation in mammals. *Q. Rev. Biol.*
705 **61**: 339-374.
- 706 Conover, D.O. & Heins, S.W. 1987. Adaptive variation in environmental and genetic
707 sex determination in a fish. *Nature* **326**: 496–498.

- 708 Conover, D.O. & van Voorhees, D.A. 1990. Evolution of a balanced sex ratio by
709 frequency dependent selection in a fish. *Nature* **213**: 577–579.
- 710 Dagg, J.L. & Vidal, S. 2004. Sex ratio adjustment and maternal condition in two aphid
711 species. *Behav. Ecol. Sociobiol.* **55**: 231–235.
- 712 Dedryver, C., Hullé, M., Le Gallic, J., Caillaud, M.C. & Simon, J.C. 2001. Coexistence
713 in space and time of sexual and asexual populations of the cereal aphid *Sitobion*
714 *avenae*. *Oecologia* **128**: 379–388.
- 715 Düsing, C. 1884. Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der
716 Menschen, Tiere und Pflanzen. *Jenaische Z. Naturwiss.* **17**: 593-940.
- 717 Fisher, R.A. 1930. *The Genetic Theory of Natural Selection*. Clarendon Press, Oxford.
- 718 Foster, W.A. 2002. Aphid sex ratios. In: *Sex Ratios: Concepts and Research Methods*
719 (I.C.W. Hardy, ed), pp. 254–265. Cambridge University Press, Cambridge.
- 720 Foster, W.A. & Benton, T.G. 1992. Sex ratio, local mate competition and mating
721 behaviour in the aphid *Pemphigus spyrothecae*. *Behav. Ecol. Sociobiol.* **30**:
722 297–307.
- 723 Frank, S.A. 1985. Hierarchical selection theory and sex ratios. II. On applying the
724 theory, and a test with fig wasps. *Evolution* **39**: 949-964.
- 725 Frank, S.A. 1987. Variable sex ratio among colonies of ants. *Behav. Ecol. Sociobiol.* **20**:
726 195–201.
- 727 Frank, S.A. 1990. Sex allocation theory for birds and mammals. *Ann. Rev. Ecol. Syst.*
728 **21**: 13-55.
- 729 Fuller, S.J., Chavigny, P., Lapchin, L. & Vanlerberghe-Masutti, F. 1999. Variation in
730 clonal diversity in glasshouse infestations of the aphid, *Aphis gossypii* Glover in
731 southern France. *Mol. Ecol.* **8**: 1867–1877.
- 732 Gilbert, N. & Raworth, D.A. 1998. Polymorphic fundatrices in thimbleberry

- 733 aphid–ecology and maintenance. *Res. Popul. Ecol.* **40**: 243–247.
- 734 Hardy, I.C.W. 2002. *Sex Ratios: Concepts and Research Methods*. Cambridge
735 University Press, Cambridge, UK.
- 736 Heie, O.E. 1980. *The Aphidoidea (Hemiptera) of Fennoscandia and Denmark I:*
737 *General Part, the Families Mindaridae, Hormaphididae, Thelaxidae,*
738 *Anoeciidae, and Pemphigidae*. Scandinavian Science Press, Klampenborg.
- 739 Inbar, M., Eshel, A. & Wool, D. 1995. Interspecific competition among phloem -
740 Feeding Insects Mediated by Induced Host - Plant Sinks. *Ecology* **76**:
741 1506–1515.
- 742 Janzen, F.J. 1994. Climate change and temperature-dependent sex determination in
743 reptiles. *Proc. Natl. Acad. Sci. USA* **91**: 7487–7490.
- 744 Janzen, F.J. & Paukstis, G.L. 1991. Environmental sex determination in reptiles: ecology,
745 evolution, and experimental design. *Quart. Rev. Biol.* **66**: 149–179.
- 746 Jiggins, F.M., Bentley, J.K., Majerus, M.E.N. & Hurst, G.D.D. 2001. How many species
747 are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common
748 by intensive sampling. *Proc. R. Soc. Lond. B* **268**: 1123–1126.
- 749 Keiser, C.N. & Mondor, E.B. 2013. Transgenerational behavioral plasticity in a
750 parthenogenetic insect in response to increased predation risk. *J. Insect Behav.*
751 **26**: 603–613.
- 752 Kindlmann, P. & Dixon, A.F.G. 1989. Developmental constraints in the evolution of
753 reproductive strategies: telescoping of generations in parthenogenetic aphids.
754 *Funct. Ecol.* **3**: 531–537.
- 755 Korpelainen, H. 1990. Sex ratios and conditions required for environmental sex
756 determination in animals. *Biol. Rev.* **65**: 147–184.

- 757 Kurosu, U. & Aoki, S. 1991. Why are aphid galls so rare? *Evol. Theor.* **10**: 85–99.
- 758 Lampel, G. 1968–1969. Untersuchungen zur Morphologie von *Pemphigus spirothecae*
759 Pass 1860 (Homoptera, Aphidoidea). *Bull. Natur. Gesell. Freiburg* **58**: 56–72.
- 760 Maynard Smith, J. 1980. A new theory of sexual investment. *Behav. Ecol. Sociobiol.* **7**:
761 247–251.
- 762 Miller, D.G. III & Aviles, L. 2000. Sex ratio and brood size in a monophagous
763 outcrossing gall aphid, *Tamalia coweni* (Homoptera: Aphididae). *Evol. Ecol. Res.*
764 **2**: 745–759
- 765 Moran, N.A. 1993. Evolution of sex ratio variation in aphids. In: *Evolution and*
766 *Diversity of Sex Ratio in Insects and Mites* (D.L. Wrensch & M.A. Ebbert, eds),
767 pp. 346–368. Chapman and Hall, New York.
- 768 Moran, N.A. & Whitham, T.G. 1990. Interspecific competition between root - feeding
769 and leaf - galling aphids mediated by host - plant resistance. *Ecology* **71**:
770 1050–1058.
- 771 Mondor, E.B., Tremblay, M.N. & Lindroth, R.L. 2004. Transgenerational phenotypic
772 plasticity under future atmospheric conditions. *Ecol. Lett.* **7**: 941–946.
- 773 Muramatsu, K. & Akimoto, S. 2016. Spatiotemporal fluctuations in natural selection
774 acting on the gall-parasitic aphid *Tetraneura sorini*. *J. Evol. Biol.* **29**:
775 1423–1436.
- 776 Ospina-Álvarez, N. & Piferrer, F. 2008. Temperature-dependent sex determination in
777 fish revisited: prevalence, a single sex ratio response pattern, and possible effects
778 of climate change. *PLoS ONE* **3**: e2837.
- 779 Podjasek, J.O., Bosnjak, L.M., Brooker, D.J. & Mondor, E.B. 2005. Alarm pheromone

780 induces a transgenerational wing polyphenism in the pea aphid, *Acyrtosiphon*
781 *pisum*. *Can. J. Zool.* **83**: 1138-1141.

782 R Core Team, 2015. R: A language and environment for statistical computing. R
783 Foundation for Statistical Computing, Vienna, Austria. See
784 <http://www.R-project.org/>.

785 Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

786 Rispe, C. & Pierre, J.S. 1998. Coexistence between cyclical parthenogens, obligate
787 parthenogens, and intermediates in a fluctuating environment. *J. Theor. Biol.*
788 **195**: 97-110.

789 Rispe, C., Pierre, J.S., Simon, J.C. & Gouyon, P.H. 1998. Models of sexual and asexual
790 coexistence in aphids based on constraints. *J. Evol. Biol.* **11**: 685–701.

791 Rispe, C., Bonhomme, J. & Simon, J.C. 1999. Extreme life-cycle and sex ratio variation
792 among sexually produced clones of the aphid *Rhopalosiphum padi* (Homoptera:
793 Aphididae). *Oikos* **86**: 254–264.

794 Sano, M. & Ozaki, K. 2011. Variation and evolution of the complex life cycle in
795 Adelgidae (Hemiptera). *Entomol. Sci.* **15**: 13–22.

796 Seger, J. & Stubblefield, J.W. 2002. Models of sex ratio evolution. In: *Sex Ratios:*
797 *Concepts and Research Methods* (I.C.W. Hardy, ed), pp. 2–25. Cambridge
798 University Press, Cambridge.

799 Simon, J.C., Rispe, C. & Sunnucks, P. 2002. Ecology and evolution of sex in aphids.
800 *Trends Ecol. Evol.* **17**: 34–39.

801 Steffan, A.W. 1970. Die eidonomischen und zytologischen Grundlagen bei der
802 Entstehung anholozyklisch-parthenogenetischer Adelgidae-Species (Homoptera:

803 Aphidina). *Zeit. Ang. Entomol.* **65**: 444–452.

804 Stouthamer, R., Breeuwer, J.A.J. & Hurst, G.D.D. 1999. *Wolbachia pipientis*: Microbial
805 manipulator of arthropod reproduction. *Ann. Rev. Microbiol.* **53**: 71-102.

806 Stubblefield, J.W. & Seger, J. 1990. Local mate competition with variable fecundity:
807 dependence of offspring sex ratios on information utilization and mode of male
808 production. *Behav. Ecol.* **1**: 68–80.

809 Tegelaar, K., Glinwood, R., Pettersson, J. & Leimar, O. 2013. Transgenerational effects
810 and the cost of ant tending in aphids. *Oecologia* **173**: 779–790.

811 Trivers, R.L. & Willard, D.E. 1973. Natural selection of parental ability to vary the sex
812 ratio of offspring. *Science* **179**: 90-92.

813 Ward, S.A. & Wellings, P.W. 1994. Deadlines and delays as factors in aphid sex
814 allocation. *Eur. J. Entomol.* **91**: 29-36.

815 Werren, J.H. 1997. Biology of *Wolbachia*. *Ann. Rev. Ent.* **42**: 587-609.

816 West, S. 2009. *Sex Allocation. Monographs in Population Biology, 44*. Princeton
817 University Press, Princeton.

818 Whitham, T.G. 1979. Territorial behaviour of *Pemphigus* gall aphids. *Nature* **279**:
819 324–325.

820 Wool, D. & Sulami, Z. 2001. Induction of alate sexuparae in root-cage colonies, and
821 female-biased sex ratios in the galling aphid, *Aploneura lentisci*. *Entom. Exp. Appl.*
822 **101**: 299-303.

823 Wrench, D.L. & Ebbert, M.A. 1993. *Evolution and Diversity of Sex Ratio in Insects*
824 *and Mites*. Chapman and Hall, New York.

825 Yamaguchi, Y. 1985. Sex ratios of an aphid subject to local mate competition with
826 variable maternal condition. *Nature* **318**: 460-462.

827

828 Figure legends

829

830 Figure 1 Life cycle of *Prociphilus oriens*. This species alternates host plants seasonally
831 between *Fraxinus mandshurica* and *Abies sachalinensis*. In autumn, sexuparae
832 (winged females) appear from the roots of *A. sachalinensis* and migrate to the
833 trunks of *F. mandshurica*. Sexuparae have matured male and female embryos in
834 their abdomen. The proportions of female embryos in broods and local
835 populations are investigated. Sexuparae usually produce bisexual broods in their
836 abdomen as shown here but sometimes produce all-female broods (abbreviated
837 as all-F sexuparae). Males (or male embryos) and sexual females (or female
838 embryos) are shown in green and orange, respectively. Each female has a single
839 egg (shown in yellow).

840 Figure 2 Yearly fluctuations in the proportions of females and sexuparae producing all
841 females (all-F sexuparae) in Bibai, Iwamizawa, and Sapporo. Data of 2002 to
842 2015 are used for comparison of the fluctuation patterns among the three
843 localities.

844 Figure 3 Relationship between the proportion of females in a year (x) and changes in
845 female proportion the next year (y). Data from three populations were pooled.
846 The regression line is represented as $y = 55.90 - 0.948 x$.

847 Figure 4 Relationship of the percentage of females that successfully produced fertilized
848 eggs to the proportion of females in arenas. The horizontal axis is represented on
849 the scale of $\log(\text{female number}/\text{male number})$. Mean percentage oviposition
850 success \pm SE is indicated. The relationship of percentage oviposition success (y)
851 to female ratio, f/m (x) is represented by the equation, $y = 8.33 x - 7.71 x^2 -$
852 1.11 .

853 Figure 5 Percentage distribution of male number per mother in each clone and in the
854 Iwamizawa population in 2008, 2009, and 2011.

855

856

857 Appendix S1 Numbers of male and female embryos each sexupara produced in her
858 abdomen. Collection localities and years are indicated.

859

860 Appendix S2 Relationship between climatic factors in a year and total maternal investment
861 and the proportions of females and all-F sexuparae in a population. Total precipitation and
862 mean temperature in July, August, and September are indicated.

Table 1 Number of sexuparae (autumnal winged females) who produced certain numbers of male and female embryos in their abdomen. Data are from 3102 sexuparae collected in Bibai from 1981 to 2015.

No. of males	No. of females												%
	0	1	2	3	4	5	6	7	8	9	10	11≤	
0	0	1	17	30	62	34	81	65	149	38	42	15	17.2
1	0	10	20	18	36	23	27	12	20	2	1	0	5.4
2	11	14	30	25	60	24	28	14	17	3	2	0	7.4
3	24	25	55	50	79	30	34	19	18	4	1	3	11.0
4	172	166	356	263	442	119	161	56	64	6	7	17	59.0

Table 2 Meteorological effects on the proportions of females and [all-F sexuparae](#) and the mean maternal investment. Stepwise multiple regression analysis was applied to these response variables by using eight explanatory variables (two locality variables and six meteorological variables at each locality). Variables indicated as ‘—’ were not adopted into the model by the backward method. * the partial regression coefficient.

Variable	Proportion of females			Proportion of all-F sexuparae			Mean maternal investment		
	Coeff.*	F	P	Coeff.*	F	P	Coeff.*	F	P
Locality (Sapporo vs others)	—	—	—	—	—	—	-0.4048	3.85	0.0555
Locality (Iwamizawa vs Bibai)	—	—	—	—	—	—	—	—	—
Precipitation, July	—	—	—	—	—	—	—	—	—
Precipitation, August	-0.0456	15.95	0.0002	-0.0671	21.32	< 0.0001	-0.0055	6.94	0.0113
Precipitation, September	—	—	—	—	—	—	—	—	—
Temperature, July	—	—	—	—	—	—	—	—	—
Temperature, August	—	—	—	—	—	—	0.5039	9.14	0.0040
Temperature, September	-2.1405	8.73	0.0048	-2.287	6.16	0.0165	-0.6838	15.55	0.0003

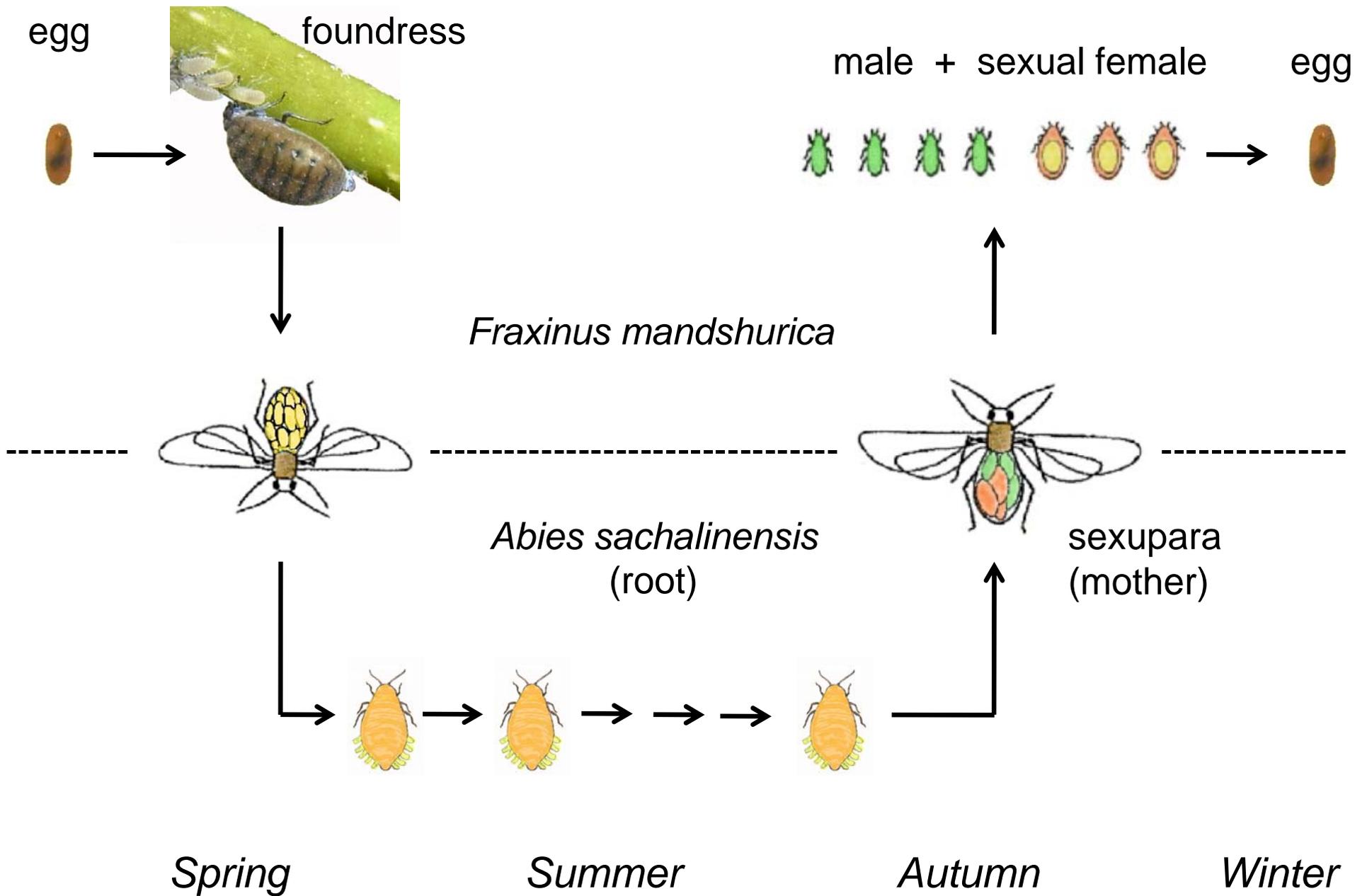
Table 3 ANCOVA for changes in the proportion of females in local populations (female proportion in year i – female proportion in year $i-1$).

Explanatory variable	df	Coefficient	F	P
locality	2	—	0.626	0.5409
% females in the previous year	1	-1.004	62.17	<0.0001
precipitation in August	1	-0.075	14.47	0.0002
temperature in September	1	-1.751	3.56	0.0675

Table 4 Numbers of sexuparae and their male and female offspring produced by colonies reared on *Abies sachalinensis* trees. The proportion of females in a colony and the mean wing length of sexuparae were also indicated. Different letters following % females show significant difference at a 5% significance level after the sequential Bonferroni adjustment. All colonies came from different clones except LY5 and LY6, which belonged to the same clone.

Colony	Year	No. sexuparae produced	Mean no. males per mother	Mean no. females per mother	% females in a colony	Mean wing length (mm) \pm SD
J	2008	86	1.84	1.84	50.00 b	3.29 \pm 0.271
L	2008	194	1.27	1.48	53.85 bc	2.95 \pm 0.185
A	2009	459	0.59	2.59	81.37 ef	3.37 \pm 0.318
B	2009	90	0.78	2.27	74.32 def	3.24 \pm 0.213
C	2009	1628	0.42	2.61	86.17 f	3.24 \pm 0.229
D	2009	215	1.34	2.33	63.40 bcd	3.26 \pm 0.318
E	2009	564	0.58	2.25	79.64 ef	3.05 \pm 0.251
G	2009	131	1.76	4.27	70.78 cde	3.58 \pm 0.178
H	2009	1021	1.36	3.39	71.38 cde	3.45 \pm 0.220
LY2	2011	692	0.64	2.82	81.50 ef	3.29 \pm 0.234
LY4	2011	758	2.94	0.68	18.78 a	3.26 \pm 0.162
LY5	2011	705	3.40	0.72	17.48 a	3.19 \pm 0.303
LY6*	2011	345	2.26	0.44	16.30 a	3.02 \pm 0.243
LY8	2011	1128	3.04	0.82	21.24 a	3.37 \pm 0.255
LY9	2011	1142	1.02	1.04	50.49 b	3.01 \pm 0.233
LY12	2011	187	2.80	3.00	51.72 b	3.59 \pm 0.229

* LY6 was excluded from the analysis of clonal differences.



- % females
- % sexuparae producing a all-female brood

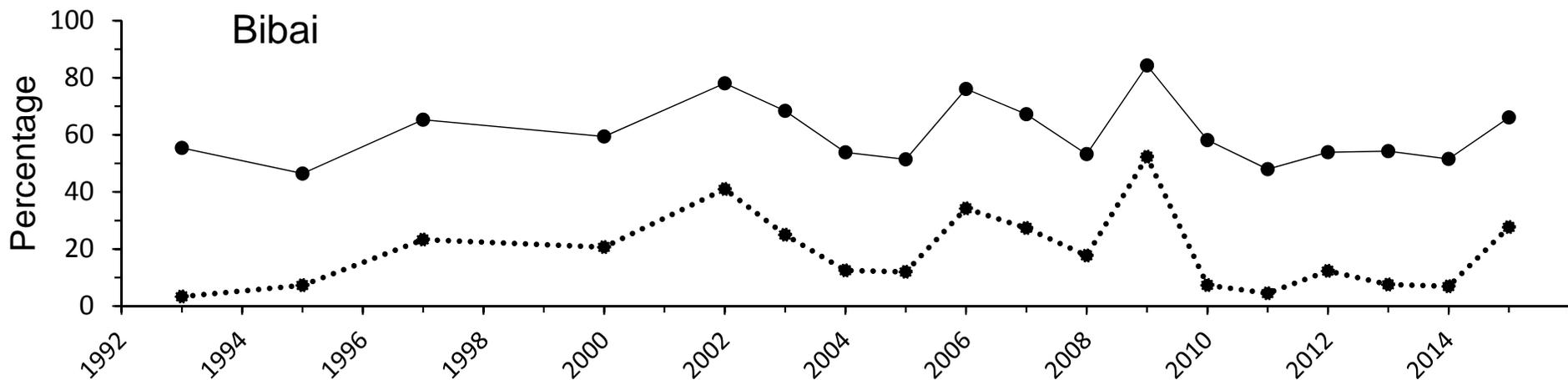
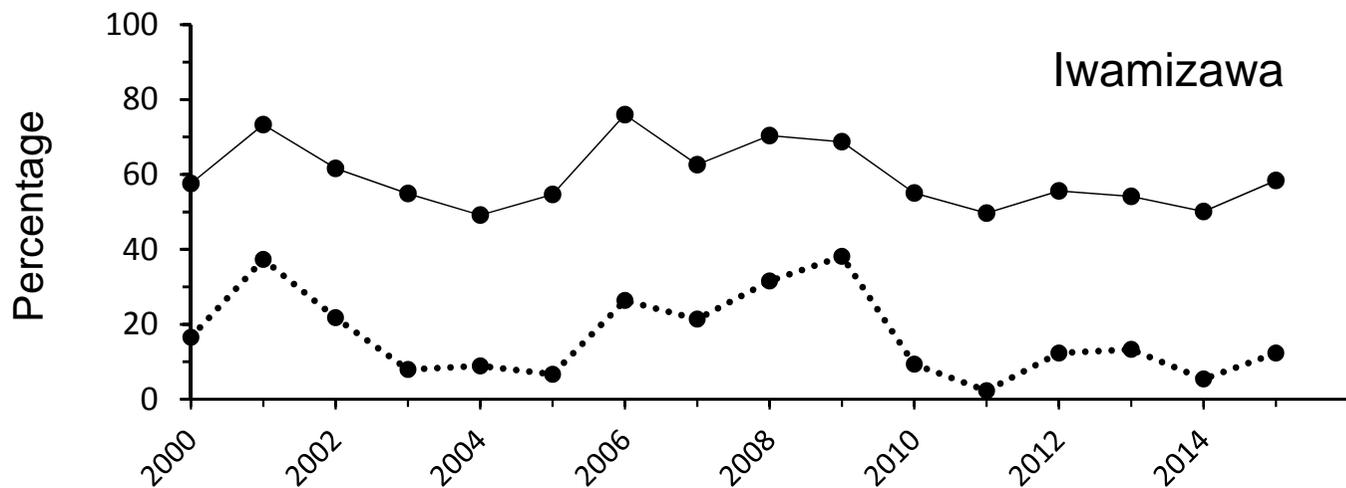
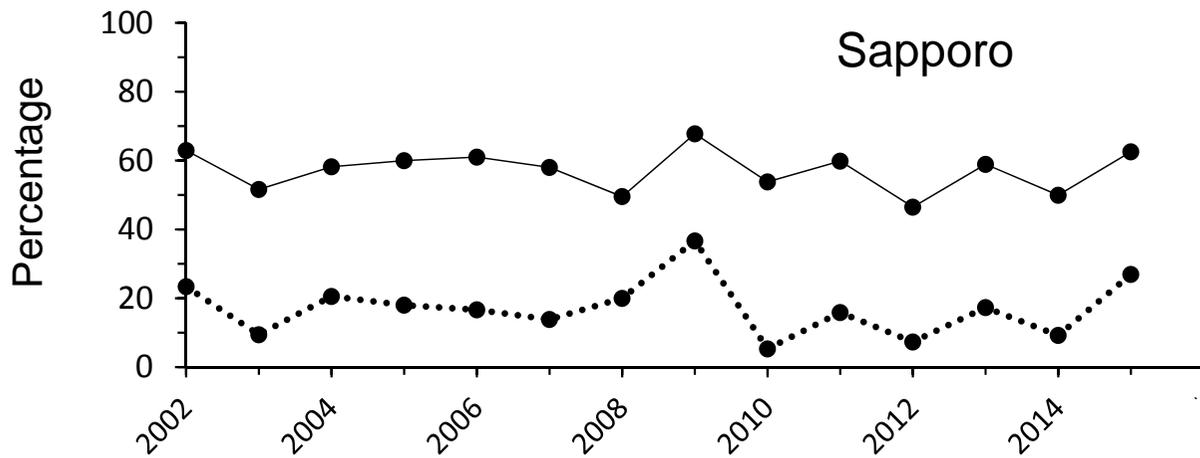


Fig. 2

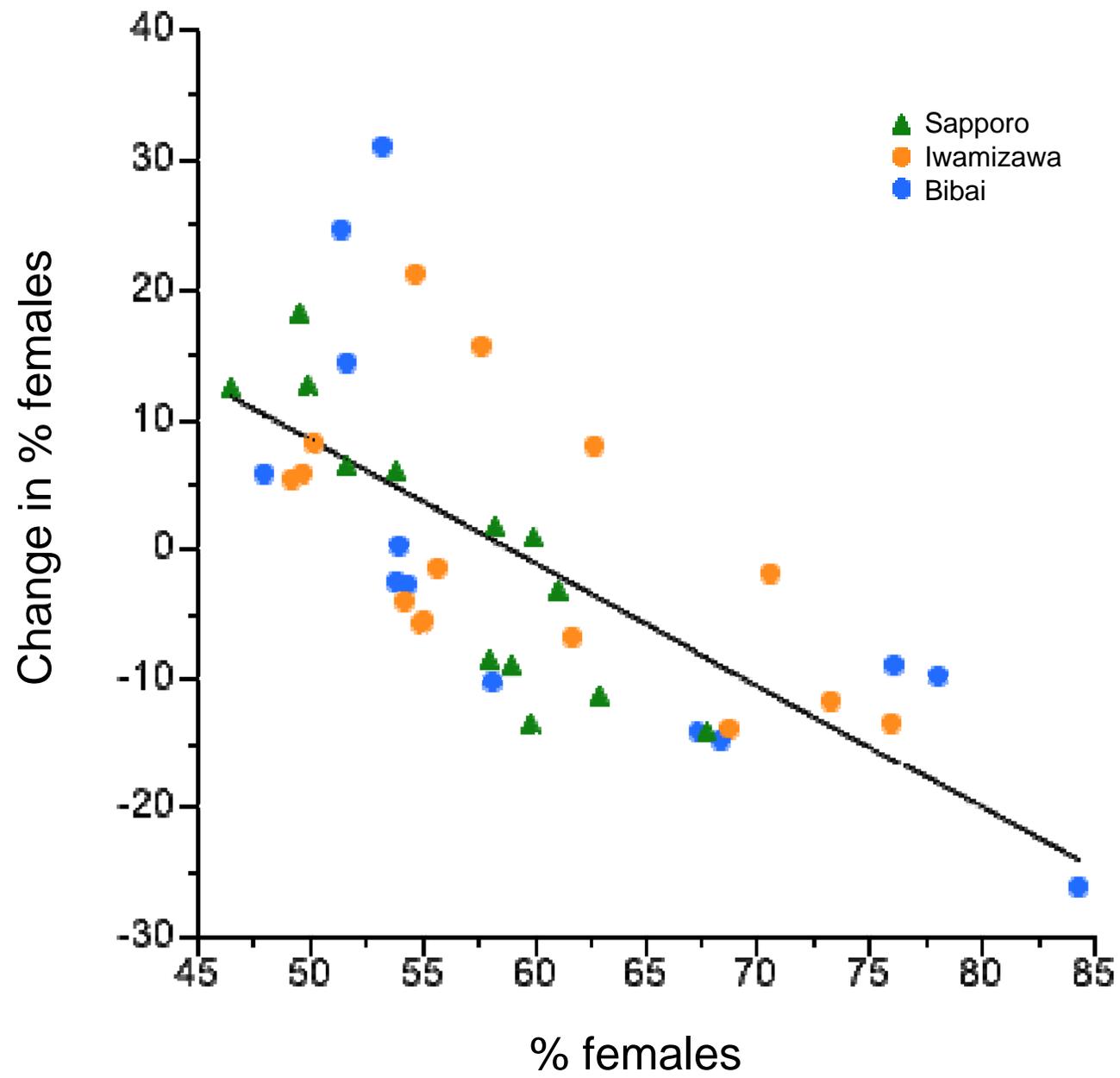


Fig.3

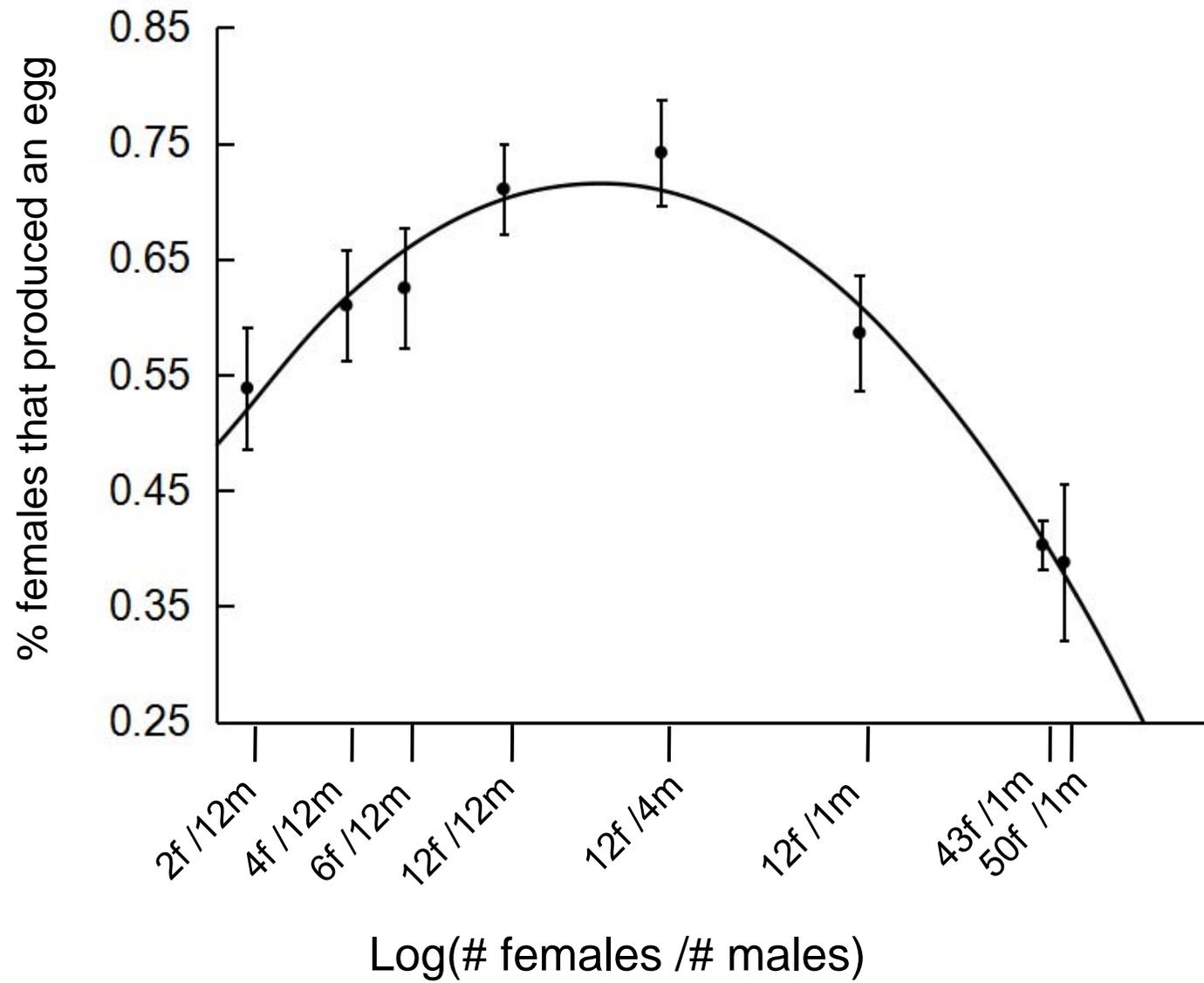


Fig.4

