1 **The difficulty of inferring process from pattern - sex ratio adaptation with helpers at the nest**

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13 **Abstract**

14 The possibility that animals can adaptively adjust their offspring sex ratio has intrigued biologists since
15 Darwin. While the population sex ratio is expected to be equal in many species, it was later also
16 hypothesised that parents should adjust their offspring sex ratio based on their situation (e.g. parental
17 condition, temperature, timing within the breeding season, territory quality, cooperative versus non-
18 cooperative breeding systems). Given that so many potential mechanisms for offspring sex ratio
19 manipulation exist, it is important to consider how to unravel the mechanism behind a sex ratio trend by
20 looking at the produced pattern. The Seychelles warbler (*Acrocephalus sechellensis*) is a textbook example
21 of a bird that is hypothesised to adaptively adjust their offspring sex ratio based on the quality of the
22 territory they reside in. In this study we used evolutionary individual-based simulations to understand what
23 kind of processes could cause a territory quality (or otherwise external) situation-dependent strategy to
24 evolve. We did so by focussing on species with helpers at the nest, and used the Seychelles warbler study
25 system as a case study. We showed that multiple mechanisms can cause the same sex ratio patterns, and
26 hence that it is difficult to disentangle these mechanisms in empirical populations.

Introduction

The possibility that animals can adaptively adjust the sex ratio of their offspring has fascinated biologists since Darwin (Darwin, 1871). The sex ratio at conception is equal (1:1) in many species, while in principle only few males are necessary to fertilise all females. Fisher's principle (formalised both by Düsing 1883 and Fisher 1930) states that this is adaptive for many species, because frequency-dependent selection favours an equal investment in male and female offspring. Yet, exceptions to this rule have also been hypothesised. Theory predicts that it can be adaptive for the offspring sex ratio to be situation dependent, and that many types of situations can cause sex ratio trends. For example, Hamilton (1967) argued that a bias in the offspring sex ratio is expected when there is a difference in dispersal tendency between both sexes, and when there is local mate competition between kin. Trivers & Willard (1973) argued that the condition of the mother could affect the fitness benefits of producing male and female offspring differently, and hence condition-dependent strategies should evolve. While Trivers and Willard mostly argued that the physical condition of the mother could influence the offspring sex ratio, it was later argued that situation-dependent offspring sex ratio adjustment could also evolve based on a condition outside of the mother, like the quality of the father (Burley, 1981; Fawcett et al., 2007), or environmental cues like the temperature (Charnov & Bull, 1989), the timing of reproduction within the breeding season (Daan et al., 1996), or the quality of the territory parents reside in (Komdeur, 1996). Additionally, for cooperatively breeding species it was hypothesised that a sex-ratio bias towards the helping sex should be expected, as the helping sex 'pays back' some of the costs of being produced (Emlen et al., 1986; Gowaty & Lennartz, 1985). Given that so many potential mechanisms exist, it is important to consider, especially for empirical studies, how to unravel the mechanism behind a sex ratio trend by looking at the produced pattern.

The Seychelles warbler (*Acrocephalus sechellensis*) is a textbook example of a bird that is hypothesised to adaptively adjust their offspring sex ratio. Seychelles warblers are facultative cooperative breeders, meaning that parents can raise offspring without help, but that part of the population shows cooperative breeding and that this help does increase the survival of offspring. In case of the Seychelles warblers, females help more often and help more efficiently. Komdeur et al. (1997) found for three years that mostly daughters were born in high quality territories, and that mostly sons were born in low quality territories. Additionally, they showed for one year that in territories where multiple helpers were already present, mostly sons were produced, independent of the territory quality. This was hypothesised to be adaptive, as daughters were produced when a helper would be beneficial (when there were enough resources to sustain a helper and also to feed newly produced offspring) and sons were produced when it was expected to be beneficial that offspring would disperse (when there were insufficient resources to sustain both a helper and new offspring, and when territories already had multiple helpers so that extra help would not benefit the offspring anymore).

In this study we used evolutionary individual-based simulations in order to understand what kind of processes could cause a territory quality (or otherwise external) situation-dependent strategy to evolve. We do so by focussing on species with helpers at the nest, and use the Seychelles warbler study system as a case study. We will demonstrate that multiple mechanisms can cause such a sex ratio pattern, and that it is difficult to disentangle these mechanisms in empirical populations. In this article we will talk about

69 (behavioural) strategies (Maynard Smith & Price, 1973) and (behavioural) reaction norms (Dingemanse et
70 al., 2010) as synonyms. They are defined as behaviours caused by genes, but that can be plastically adjusted
71 based on environmental cues.

72

73

74 **The model**

75 <u>Overview</u>

76 We consider an individual-based model for the evolution of offspring sex ratio strategies. The simulation
77 progresses in discrete time steps, with each time step corresponding to a breeding season. There are two
78 important timescales, a within-season 'ecological' timescale in which all behaviours occur, and an across-
79 season 'evolutionary' timescale, on which the frequency distribution of the inherited reaction norms
80 changes due to a combination of natural selection, mutation and genetic drift. Individuals in our model can
81 survive for multiple seasons, hence there are overlapping generations. Our model consists of territories
82 that each have a specific quality that indicates the available resources of the territory at the beginning of
83 a breeding season. Territory qualities differ between territories, but stay constant over time for each
84 territory. Territories can contain a breeding female, a breeding male, helpers and offspring. Additionally,
85 some individuals are not affiliated with specific territories and are referred to as "floaters". Every individual
86 has a status (breeder, helper, chick, floater), a sex, an age, genes to determine their genetic offspring sex
87 ratio strategy, and a level of gathered resources within a season. During the breeding season, one offspring
88 is produced per territory (and thus per pair of breeders). The genotype of the offspring results from the
89 genotypes of its parents in line with Mendelian inheritance, but with a small chance of mutation. The sex
90 of the offspring is determined on the basis of the sex-ratio strategy of the mother, which depends on the
91 genes of the mother, the territory quality the mother resides in and in some simulations also on the number
92 of helpers within the territory. Offspring are provisioned with resources. The amount of resources fed to
93 offspring depends on the available resources within a territory, on the number of adults in the territory
94 (the number of helpers can vary) and on the sex of these adults. Next, survival is checked. For adults,
95 survival depends on a constant parameter, while offspring survival depends on the amount of resources
96 they acquired. After all non-surviving individuals are removed from the population any available breeding
97 positions are filled in by either helpers, floaters or chicks. Afterwards, the remaining chicks will either stay
98 on their natal territory to become a helper or disperse and become a floater. Over the breeding seasons
99 on the 'evolutionary' timescale, strategies leading to a higher reproductive output will increase in
100 frequency and replace less successful strategies.

101

102 <u>Model implementations</u>

103 We implemented the model in different ways. First, we set all general parameters based on the data from
104 the Seychelles warbler (see Supplement 1 for a description of all the relevant ecology of Seychelles
105 warblers). In this case, we checked for an evolving sex ratio strategy based on territory quality, and in
106 another model we also checked for a strategy based on territory quality and the number of helpers, both
107 as in Komdeur et al. (1997). In the Seychelles warbler scenario there are three key differences that could
108 affect sex ratio and dispersal patterns. First is a difference in the probability to stay on the natal territory
109 between sons and daughters. Second is a difference in helping efficiency between male and female helpers

110   (i.e. offspring that has stayed), and third is a benefit of philopatry, which we expressed as a difference in
111   the probability of obtaining a breeding position between helper and floater birds. In our other model
112   implementations we used the simplest case and made the sex ratio strategy depend only on territory
113   quality. Moreover, we investigated these three main differences one at a time to understand their separate
114   effects on the evolution of sex ratio patterns. Lastly, as we found that the probability to stay or leave the
115   natal territory has a large effect on sex ratio patterns, we studied additional model implementations where
116   the probability to stay co-evolved with the probability to produce a son. We used two methods for this:
117   First, the probability to remain philopatric was caused by one gene that was bound between 0 (always
118   leave) and 1 (always stay). Second, we made the probability to stay dependent on the territory quality, in
119   a similar way as the probability of producing a son. For all parameter values and model implementations
120   used, see table 1.

121

122   <u>Territories and individuals</u>

123   The results below are based on simulations of a system with 1000 territories, resulting in an average
124   population size of ca 3800 individuals to ensure that natural selection dominates genetic drift. Each
125   territory started at the beginning of a season with a number of resources ranging from 40 to 140 in arbitrary
126   units (for a justification for these values see Supplement 1), which was initialised randomly using a uniform
127   distribution. These start resources were translated into a territory quality that ranged from -1 to 1, so that
128   the genetic parameters had easily translatable values and so that mutations had a similar effect on all
129   genetic parameters.

130   Individuals in the simulation were haploid and carried genes that influence their offspring sex ratio (in case
131   of breeding females), and their staying probability (in the case of chicks) for the model implementations
132   where this was an evolvable trait. Populations were initialised as monomorphic, so that every individual
133   carried identical genes. Genes were initialised so that at first an equal sex ratio was expected, and a 50%
134   chance of remaining philopatric was expected.

135

136   <u>Behavioural strategies</u>

137   In our model, we explored two different approaches for calculating the probability of producing a son. In
138   the first approach, the probability to produce a male offspring (M) was influenced only by the quality of
139   the territory (Q) occupied by the breeding female:

140    $$M(Q) = \frac{1}{1 + e^{-\beta * (Q - \alpha)}} \tag{1}$$

141   Here, α and β are heritable traits. The value of α indicates the territory quality where M will be 0.5, and β
142   is the slope (in point α) of the effect of territory quality on the probability of producing a son, and thus
143   indicates the strength and direction of the relationship between territory quality and the probability to
144   produce male offspring. For example, when β is positive more male offspring are produced in high quality
145   territories and more female offspring in low quality territories.

146   The second approach was used for our second Seychelles warbler case study, where it was hypothesised
147   that the offspring sex ratio also depends on the number of helpers. For this second approach, we made M

148 also dependent on the number of helpers by using different genes (α_i and β_i) for each level of number of
149 helpers. In this case, the number of helpers was divided in three categories (as described by Komdeur et
150 al., 1997): no helpers, one helper, or two or more helpers.

151 We used a non-standard parameterisation of M (usually the exponent of -β*Q-α is taken), as this allows
152 for a clearer biological interpretation of α and β. Moreover, eq. 1 is a logistic function, which we chose to
153 represent M because sex ratio data in empirical systems is often analysed using a logistic regression model.
154 Furthermore, in these empirical studies the sex ratio is often described as the proportion of sons (instead
155 of a ratio), which is also what we use in our study both for the realised sex ratios as well as for the genetic
156 reaction norms. In the result section we generally will present the reaction norms of the probability to
157 produce a son (and not the realised sex ratio trends) when both show the same patterns, because more
158 information can be obtained from the genetic strategies. In our simulations, the calculated probability to
159 produce male offspring M was used as the mean of a Bernoulli distribution, from which the sex of the
160 offspring was determined.

161 As described above, in an extension of our model, we let the probability of chicks to remain philopatric and
162 become a helper on the natal territory (versus becoming a floater) also evolve. Here, every individual has
163 sex-specific genes to determine their probability to stay. These genes were initialised in such a way that
164 half of the offspring remained philopatric, and in such a way that the population started monomorphic.
165 We implemented this evolved philopatry probability (P) in two ways: 1). As a single gene with a value
166 between 0 and 1, and 2). in a way where the probability to remain philopatric was dependent on the
167 territory quality, using a similar formula as that for M:

168
$$P(Q) = \frac{1}{1 + e^{-\delta*(Q-\gamma)}} \qquad\qquad (2)$$

169 Here γ and δ are heritable traits. The value of γ indicates the territory quality where P is 0.5, and δ indicates
170 the strength and direction of the effect of territory quality on the probability of a chick to stay.

171

172 <u>Reproduction and inheritance</u>

173 Reproduction occurs when both a breeding male and breeding female are present on the territory. If not
174 enough adult individuals of the correct sex exist in the population, breeding positions stay empty on some
175 territories and reproduction cannot occur on those territories. In our simulation parents are capable of
176 producing only one offspring per season. Offspring inherit genes from either their father or mother with
177 equal probability. Genes that are involved in expressing the same trait, e.g. those involved in determining
178 the offspring sex ratio when 1 helper is present, are genetically linked and thus inherited together.
179 Conversely, genes governing different traits are not linked. Genes can mutate with a mutation probability
180 of 0.05, and mutate with a mutation step size which is drawn from a normal distribution with a mean of 0
181 and a standard deviation of 0.01. The mutation rate was chosen a little bit higher than strictly conservative
182 values (0.05 instead of 0.01), to speed up evolution and not cause any computational issues. However, it is
183 important to note that therefore some variance between individuals in gene values are expected, so part
184 of the between-individual variance in strategies is caused by this choice.

185

186   <u>Resource dynamics</u>

187   Within each territory, resources were initially allocated among all adults, including breeding individuals
188   and potential helpers. It was checked whether enough resources were available for each adult to reach a
189   sufficient level to start feeding offspring. If insufficient resources were present, the adults would consume
190   everything and the offspring would receive no resources. If sufficient resources were available to start
191   provisioning offspring, the remaining resources could be allocated to the chick. The maximum amount of
192   resources each adult could provide to the offspring was a set parameter, which we call helping efficiency,
193   and which depended on the status and sex of adults. This helping efficiency was based on estimated
194   provisioning rates of Seychelles warblers (Richardson et al., 2003), which found that breeders and female
195   helpers all provisioned at equal rates (7 visits/hour), but that male helpers provisioned less (3 visits/per
196   hour). In our model, helping efficiency reflects the provisioned resources per season, but we assumed this
197   to have the same ratios. If there were enough resources available on the territories in the model, each
198   adult provided the maximum they could, but if there were less resources available, these were all provided
199   to the chick. Lastly, we assumed that after a certain level (35 resources) chicks could not consume more,
200   even if there were enough resources and helpers available.

201

202   <u>Survival</u>

203   For adults, the probability to survive was a fixed parameter (see Supplement 1). For offspring, the survival
204   probability from one breeding season to the next depended on their acquired amount of resources R after
205   resource distribution. Offspring survival probability was calculated using:

206    $$S(R) = \frac{R^2}{R^2 + R_{50}{}^2}$$ (3)

207

208   Here, R represented the realised resource level each individual chick acquired and R_{50} was a constant. The
209   value of R_{50} signifies the resource threshold at which the survival probability is 50%. As resources were
210   'consumed', they were not carried over to the subsequent season.

211   In the Seychelles warblers, survival from fledging to the next year is estimated to be ~60% (Brouwer et al.,
212   2006). Hence, we assumed that survival from hatching to the next year would be lower. Moreover, helpers
213   have a positive influence on the survival of chicks, but Komdeur et al. (1997) hypothesised that this benefit
214   levelled off after two helpers. Therefore, we set the parameter R_{50} to 18 so that survival of chicks on
215   territories without any helpers was below 50% (to be precise 38%), while survival with a female helper was
216   above 50% (to be precise 58%), and that a second female helper would improve survival considerably (to
217   71%), while a third helper would not add as much benefits anymore (to 78%), and every additional helper
218   would increase survival with even smaller steps. However, these estimates are only true for territories with
219   enough resources, as helpers also all would consume 20 resources before any feeding towards the chicks
220   was performed, hence additional helpers could also decrease the survival of offspring to 0%. Therefore,
221   the benefits of helpers depended on the territory quality and thus a potential territory quality dependent
222   sex ratio strategy could give evolutionary benefits.

223

Status changes

225 After survival, individuals could change their social status. First, the existence of vacant breeding positions,
226 caused by mortality of previous breeders, was checked. If vacant spots were identified, other individuals
227 could occupy these positions. Empty breeding spots were filled in order of territory quality, where
228 territories with highest quality were filled first. Any available helpers, floaters and offspring of the correct
229 sex (of the available breeding position), could become the new breeder. The replacer was chosen using a
230 weighted lottery, taking into account the number of individuals in each category as well as a specific
231 weighing factor for each category. The weighing factor of chicks was always lower than that of helpers and
232 floaters (in the results shown below this weighing factor was 0.2). In most of our models, the weighing
233 factor for helpers and floaters was equal (each 0.4), but in our Seychelles warbler case study we wanted to
234 reflect potential benefits of philopatry (see Supplement 1 for details), and hence helpers had a higher
235 probability of becoming a breeder than floaters (with a weighing factor of 0.5 for helpers and 0.3 for
236 floaters).

237 Next, the remaining offspring could either stay on their natal territory and become a helper, or they could
238 leave and become a floater. The probability to stay depended on the chick's sex. This probability could
239 either be a fixed value per sex, or was determined by genes and the quality of the natal territory, as
240 described above. For both cases the actual action of staying versus leaving was determined by drawing
241 from a Bernoulli distribution with the staying probability as a mean.

242

243 *Table 1: Parameter values in our simulations. The default value of each parameter is depicted in bold.*

Parameter name	Parameter Implementations
The probability to produce male offspring (M) depends on	- **Territory quality** - Territory quality and number of helpers
Resources consumed per adult before feeding chicks	**20**
Helping efficiency of breeders	**7**
Helping efficiency of female helper	**7** 3 0
Helping efficiency of male helper	**3** 7 0
Maximum resource consumption for chicks	**35**
The weighted lottery weight of a helper to become breeder	0.5 **0.4**
The weighted lottery weight of a floater to become a	0.3

breeder	**0.4**
The weighted lottery weight of a chick to become a breeder	**0.2**
The philopatry probability (P) of a female chick	**0.7** 0.3 0.5 Evolving parameter between 0 and 1 Situation-dependent strategy
The philopatry probability (P) of a male chick	**0.3** 0.7 0.5 Evolving parameter between 0 and 1 Situation-dependent strategy

244

Technical note

246   Simulations were conducted using C++ in Visual Studio (*Visual Studio Community*, 2022, version 17.4.4).
247   Figures were generated using R version 4.2.2 (R Core Team, 2022), utilising the ggplot2 (Wickham, 2016)
248   and cowplot (Wilke, 2020) packages.

249

Results

251

252   <u>Seychelles warbler case study</u>
253   In Figure 1 we show the results for the Seychelles warbler case study with a sex ratio strategy depending
254   on territory quality. A situation-dependent sex ratio strategy evolved where more females were produced
255   in high-quality territories and more males were produced in low-quality territories. Mostly, one strategy
256   evolved with some variance between individuals (e.g. Fig. 1a), while in some cases for short periods two
257   distinct polymorphisms evolved (e.g. Fig. 1b). Yet, these polymorphisms always disappeared quickly. The
258   equilibrium for the slope (β) seems to never be reached. Once the slope is strongly negative, selection
259   becomes weak as making the slope more negative only results in very small phenotypic changes. Hence, at
260   this moment drift becomes stronger than selection and thus the exact value of the slope varied over time
261   and between replicates (Fig. 1c,d). When we changed the parameters so that males had a higher staying
262   probability and a higher helping efficiency (i.e. the opposite of the Seychelles warbler case), an opposite
263   curve evolved where more sons were produced in high quality territories and more daughters in low quality
264   territories.

265

266   We also simulated the possibility of a situation-dependent sex ratio trend based on territory quality and
267   helper presence (Fig. 2). In this case, a strategy evolved where more daughters were produced in high
268   quality territories, while more sons were produced in territories with low quality and in territories where
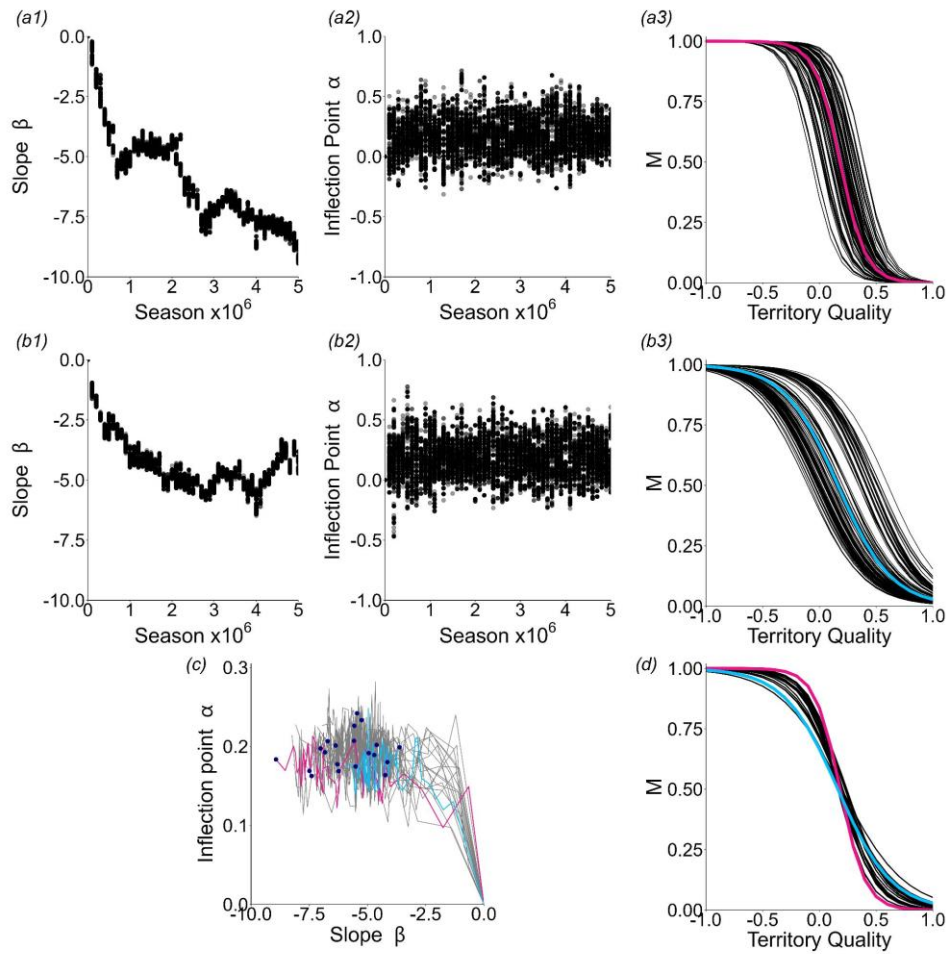
Figure 1: The evolution of a situation-dependent sex ratio strategy based on parameters from the Seychelles warbler. (a) and (b) show two different replicates of the same simulation. (a1) and (b1) represent the genetic values of the slope (β) of the reaction norms over time. Each dot represents the value of one breeding female. Over time the slope becomes negative. Sometimes polymorphisms occur, but these disappear again relatively quickly. Also note that an equilibrium is not reached, as at some point drift becomes stronger than selection. (a2) and (b2) represent the genetic values of the inflection point (α) of the reaction norms over time. Each dot represents the value of one breeding female individual. There are individual differences in inflection point, but the mean value stays more or less constant over time. (a3) and (b3) represent the individual genetic sex ratio reaction norms (M) of each breeding female at the end of the simulation. The pink and blue lines show the population average sex ratio strategy of the two replicates, respectively. (c) shows for 20 replicates how the the values of the average slope (β) and inflection points (α) changed over time. Each simulation started in (0,0) and ended in one of the dark blue dots. The replicates shown in (a) and (b) are represented by the same pink and blue as used in (a3) and (b3), respectively. (d) shows the population average genetic sex ratio reaction norms of these 20 replicates at the end of the simulation. The pink and blue line represent the replicates shown in (a) and (b), respectively.
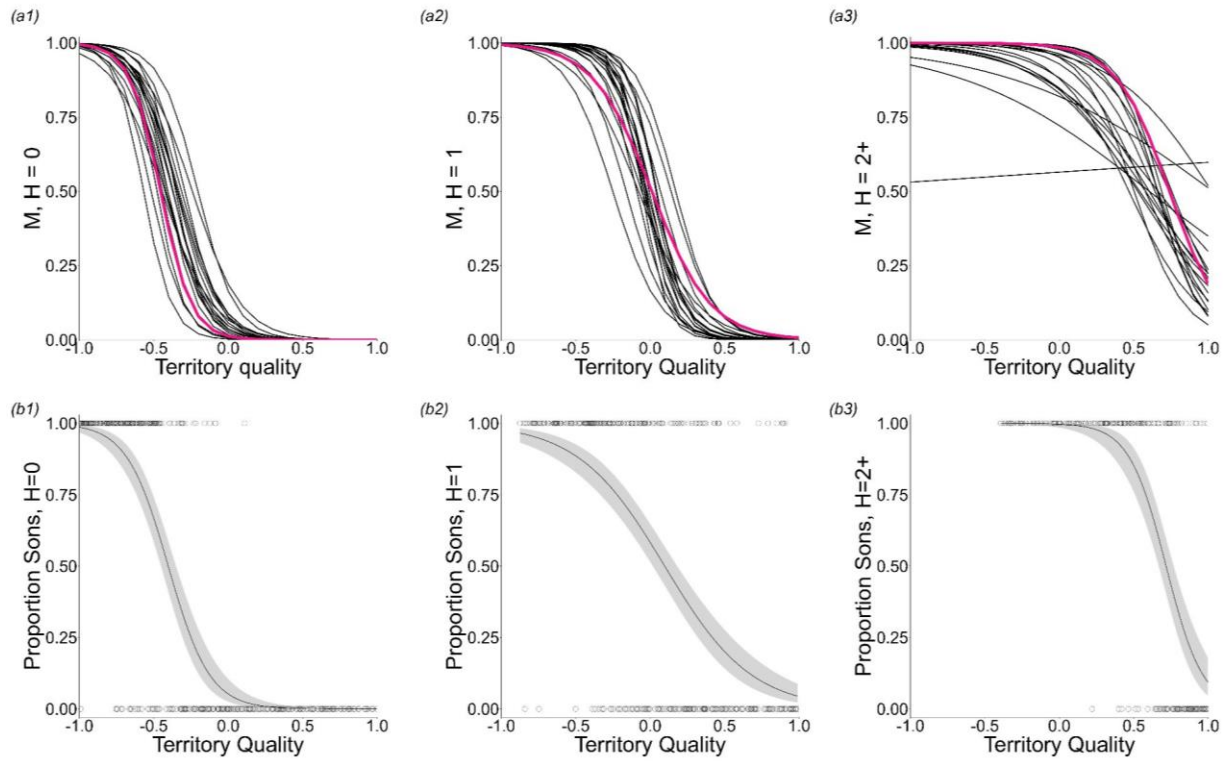
Figure 2: The evolution of a situation-dependent sex ratio strategy based on territory quality and number of helpers, and based on parameters from the Seychelles warbler. (a) The average population sex ratio reaction norms of 20 replicate simulations at the end of the simulation. The average reaction norms for 0 helpers (a1), 1 helper (a2) and 2+ helpers (a3) are shown. The inflection points (α) of these curves move to the right with more helpers. There is more variation in the curves with 2+ helpers (a3), because there are fewer territories with this many helpers, hence there is less selection on these curves. (b) the realised proportion of sons produced in a single replicate (the replicate coloured in pink in (a)). Each dot represents a single offspring and the lines plus confidence intervals show the best fitting logistic curves through these points (as one would do with empirical data). Note that the curves in (b) look similar to the genetic reaction norm in (a), but no territories with a low quality and 2+ helpers existed that also produced offspring, and hence this part of the behavioural reaction norm is never expressed.

270   independent, they evolved similar slopes. Number of helpers mainly affected the inflection points of the
271   curves, where these inflection points moved to the right with more helpers. Moreover, we found more
272   variation in curves for the situation of 2+ helpers, because there were fewer territories with 2+ helpers and
273   hence there was less selection on these curves. Lastly, we found that in some cases deviations between
274   genetic reaction norms and realised sex ratio strategies appeared. For example, territories with low quality
275   sometimes did not have enough resources to obtain 2+ helpers or to produce surviving offspring when 2+
276   helpers were present. Hence, this part of the genetic reaction norm for 2+ helpers was never under direct
277   selective pressures.

278   Causes of sex ratio patterns
279   As previously mentioned, there are three major factors that could affect or cause the evolved sex ratio
280   strategies in the Seychelles warbler case study. First, there is a difference in helping efficiency between
281   male and female helpers. Second, there is a difference in staying probability (i.e. stay to become a helper
282   versus disperse to become a floater) between sons and daughters. Last, there is a difference in the
283   probability of helpers and floaters to obtain a breeding position, which indirectly could affect sex ratio
284   strategies. In the next sections we studied their separate effects on the evolution of sex ratio strategies.
285
286   *Equal staying probability, no helping*
287   As a control, we set the staying probability for males and females to the same level (0.5) and reduced the
288   helping efficiency to 0 for both sexes. We also made the probability to become a breeder equal for helpers
289   and floaters. Here, we found that sex ratio strategies still evolved (Fig. 3A). In some cases, condition-
290   dependent strategies based on territory quality evolved, both in the direction where more males were
291   produced in high quality territories as well as in the direction where more females were produced in high
292   quality territories. Interestingly, in some replicates no environmental situation dependent sex ratio
293   strategy seemed to have evolved on the population level (i.e. Fig. 3A2 shows flat lines), but there were
294   many different situation-dependent sex ratio strategies on the individual level, that overall resulted in a
295   flat line on the population level (See Supplement). Additionally, in some replicates polymorphisms evolved
296   where part of the population would always produce sons and the other part would always produce
297   daughters, or a mix between such a polymorphism and a territory quality dependent sex ratio strategy.
298   Again, these strategies resulted in a flat line on the population level and hence overall it is not apparent
299   that a sex ratio strategy has evolved. As none of the three factors differed in these simulations, we assume
300   that sex ratio patterns evolved in this case firstly due to mutation and stochastic effects. Once part of the
301   population started to have a skew in their sex ratio strategy (based on territory quality or not), it became
302   beneficial for the other part of the population to skew their sex ratio in the opposite direction due to
303   Fisher's principle. Consequently, this can then lead to strong sex ratio strategies even though there was no
304   apparent reason for them to evolve.
305
306   *Difference in helping efficiency and in staying probability*
307   As a second control, we reran the Seychelles warbler case study (higher helping efficiency for females: 7 vs
308   3; and a higher staying probability for females: 0.7 vs 0.3), but without a difference in the probability to
309   become a breeder between helpers and floaters. Here again in all 20 replicates a sex ratio strategy evolved
310   where mostly daughters were produced in high quality territories and mostly sons were produced in low
311   quality territories (Fig. 3B). There seemed to be no big difference between the 20 replicates with a
312   difference in breeding probability between breeders and floaters and these 20 replicates without that
313   difference. Therefore, it seems that the breeding probability had little to no direct effects on the evolution
314   of sex ratio strategies.
315
316   *Difference in helping efficiency*
317   Here, we implemented only a difference in helping efficiency, so that females helped more than males (7
318   vs 3 resources), and there was an equal probability of staying for both sexes (0.5), and no difference in the
319   probability to obtain a breeding position between helpers and floaters. Again, a sex ratio strategy evolved

320
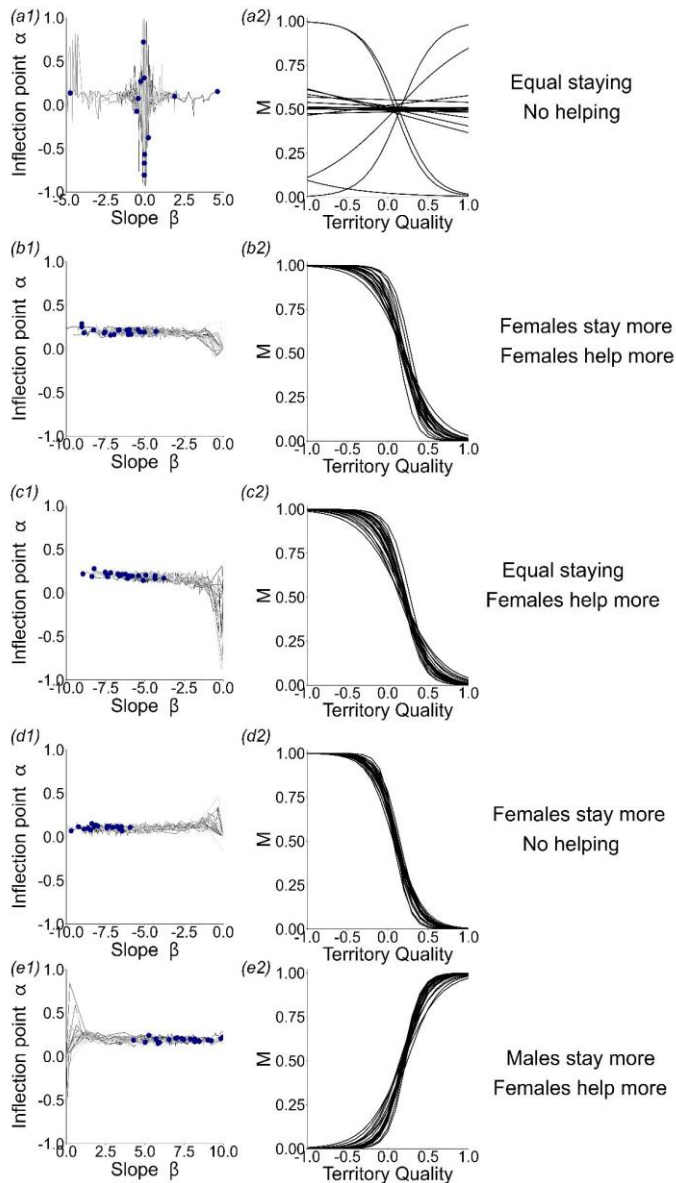
Figure 3: The evolution of condition-dependent sex ratio strategies under multiple scenarios. Parts 1 (left column) of each scenario shows the population averages of slopes (β) and inflection points (α) for 20 replicates over time, as in figure 1c. Each replicate started in (0,0) and ended in a dark blue dot. Parts 2 (right column) of each scenario shows the average population sex ratio reaction norms of the 20 replicate simulations at the end of the simulation (as in figure 1d). For all the scenarios shown in this figure there was no difference in the probability to become a breeder between helpers and floaters. (a). A scenario with equal probability to stay between males and females (0.5) and neither sex helped (helping efficiency of 0). (b) Females had a higher probability of staying than males (0.7 vs 0.3) and helped more efficiently (helping efficiency of 7 vs 3, as in figure 1, but with equal breeding probabilities for helpers and floaters). (c) Females had a higher helping efficiency (7 vs 3), and both sexes stayed with an equal probability (0.5). (d) Females had a higher staying probability (0.7 vs. 0.3), but neither sex helps (helping efficiency 0). (e). Males had a higher staying probability (0.7 vs 0.3), while females had a higher helping efficiency (7 vs 3).

321 (Fig. 3C). Hence, we expect in this case that a sex ratio strategy evolved because there is a benefit for high-
322 quality territory parents to produce daughters as these have a higher probability of becoming a helper.
323 Moreover, for low-quality territory parents it was likely beneficial to produce sons, not because these
324 would disperse (as the staying probability was equal between the sexes), but because of the rarer-sex effect
325 of Fisher's principle.
326
327 *Difference in staying probability*
328 Next, we implemented only a difference in staying probability, where females had a higher staying
329 probability than males (0.7 versus 0.3). In this case neither of the sexes helped once they stayed (i.e. helping
330 efficiency was 0), and the probability to become a breeder was equal for helpers and floaters. Once more
331 a sex ratio strategy evolved where mostly daughters were produced in high quality territories while sons
332 were produced in low quality territories (Fig. 3D). We expect that in this case the strategy evolved because
333 there is a benefit for low-quality territory parents to produce sons, as these have a higher probability of
334 dispersing and therefore in the next season will not consume resources that could be used to feed new
335 offspring. Furthermore, for high-quality territory parents it was likely beneficial to produce daughters, not
336 because these daughters would help, but because of Fisher's principle. We ran both the difference in
337 helping efficiency model and the difference in staying probability models with and without a difference in
338 the probability to become a breeder between helpers and floaters, and again this did not qualitatively
339 affect the results.
340
341 *Higher staying probability for males, higher helping efficiency for females*
342 Subsequently, we switched the staying probabilities, so that males had a higher staying probability than
343 females (0.7 vs 0.3), but we kept the difference in helping efficiency the same as before, so that females
344 had a higher helping efficiency than males (7 vs 3). Again, helpers and floaters had an equal probability to
345 become a breeder. In this case, a sex ratio strategy evolved in the opposite direction as before, so that
346 mostly males were produced in high quality territories while mostly females were produced in low quality
347 territories (Fig. 3E). Thus, for these specific parameter values, the staying probability had a stronger effect
348 on the evolution of the sex ratio strategy than the helping efficiency. In other words, this curve likely
349 evolved because low-quality territory parents benefitted from producing the dispersing sex (in this case
350 females). High-quality territory parents conversely produced the opposite sex (in this case males), because
351 of Fisher's principle, even though producing females could have resulted in more efficient helpers in these
352 high quality territories.
353
354 <u>Evolved staying probability</u>
355 As the staying probability had an important effect on the evolution of adaptive sex-ratio strategies, we also
356 investigated a situation where the staying probability and a sex ratio strategy could co-evolve. As described
357 previously, we implemented the evolution of a staying probability in two ways. First, let the staying
358 probability be a situation-independent trait governed by two genes (one for each sex), and second, the
359 staying probability was based on sets of evolving sex-specific genes and could depend on the territory
360 quality, in a similar way as the sex ratio strategy (eq. 1). Contrary to the previous scenario, in both cases
361 we found that the breeding probability of helpers and floaters had a big effect on the results.
362
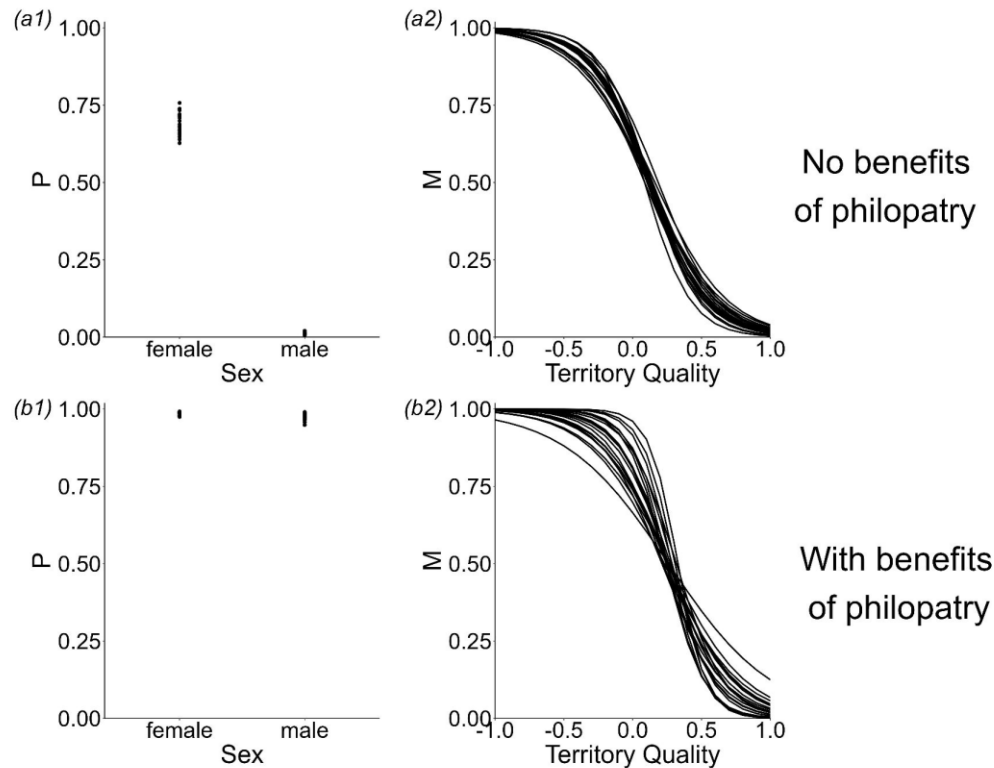
Figure 4: The effect of breeding probability on the co-evolution of a situation-dependent sex ratio strategy and a situation independent staying probability. Here, the probability of philopatry (P) evolved as a single gene . (a1) and (b1) show the population average gene values of the probability of males and females to stay on their natal territory for 20 replicates. (a2) and (b2) show the population average strategies to produce a son for 20 replicates. (a) represents simulations where there was an equal probability for helpers and floaters to become a breeder, while (b) represents simulations where helpers had a higher probability of becoming a breeder than floaters.

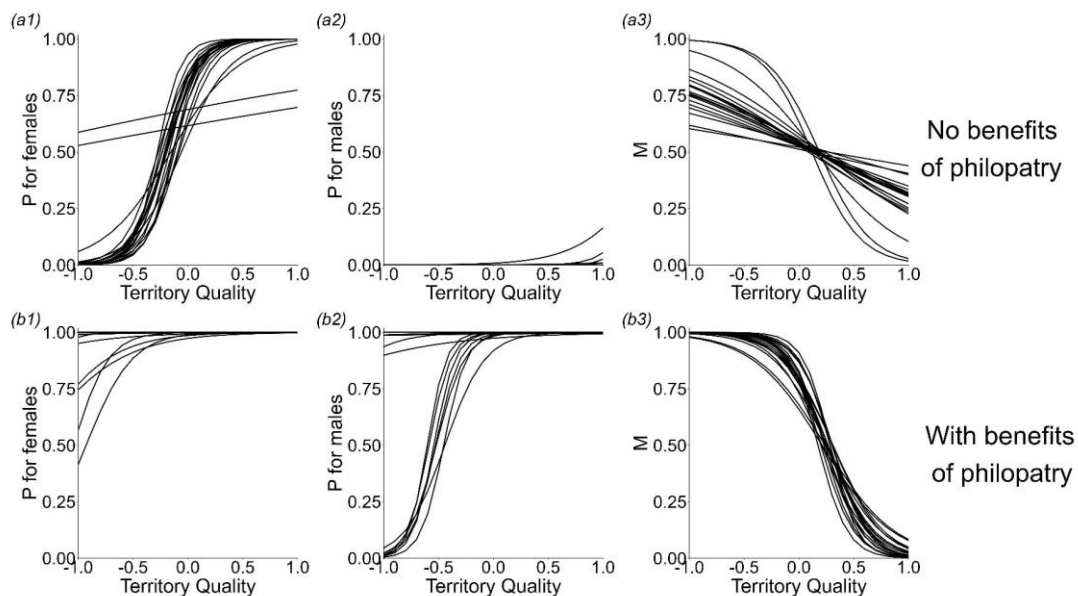363 *Staying probability as a situation-independent strategy*
364 In the first case, the staying probability co-evolved with the sex ratio strategy, females had a higher helping
365 efficiency (7 vs 3) and there was no difference in breeding probability between helpers and floaters. Here
366 (Fig. 4A), females evolved to stay with a high probability (around 70%), while males evolved to stay with a
367 very low probability (around 0%). In this case, again a sex ratio strategy evolved where mostly daughters
368 were produced in high quality territories and mostly sons were produced in low quality territories. We
369 expect that this sex ratio strategy evolved because it was beneficial for low-quality territory parents to
370 produce sons (the dispersing sex), while it was beneficial for high-quality territory parents to produce
371 daughters, because of Fisher's principle and because daughters were more efficient helpers. Once we
372 included a difference in breeding probability between helpers and floaters (so that helpers had a higher
373 chance of becoming breeders) the results changed. Here (Fig. 4B), both females and males evolved a
374 staying probability of around 100%. Again, a sex ratio strategy evolved where mostly daughters were
375 produced in high quality territories and mostly sons in low quality territories. However, in this case, we

376 expect that this sex ratio strategy evolved because it was beneficial for high-quality territory parents to
377 produce daughters (as females were more efficient helpers), while low-quality territory parents produced
378 more sons likely because of Fisher's principle.
379
380 *Staying probability as a territory quality situation-dependent strategy*
381 We also implemented an evolved staying probability as a situation-dependent variable, where sex-specific
382 plastic reaction norms based on territory quality could evolve, similarly as the sex ratio strategy. Again, in
383 the first case females had a higher helping efficiency (7 vs 3) and there was no difference in breeding
384 probability between helpers and floaters. Here (Fig. 5A), females evolved a situation-dependent staying
385 strategy, where they mostly stayed in high quality territories, while they mostly left in low quality
386 territories. Males evolved a strategy where they almost always leave, although in high quality territories
387 their staying probability became a bit higher than 0. A sex ratio strategy evolved where daughters were
388 produced in high quality territories and sons in low quality territories, but in some replicates the slope of
389 this strategy seemed less steep than in all other cases. We expect this strategy evolved because it is
390 beneficial for low-quality territory parents to produce the dispersing sex (sons), while for high-quality
391 territory parents it is beneficial to produce the opposite sex (daughters) due to Fisher's principle and
392 because daughters were more efficient helpers. Again, once we included a difference in breeding

Figure 5: The effect of breeding probability on the co-evolution of a situation-dependent sex ratio strategy and a situation-dependent staying probability. (a1) and (b1) show the population average strategy of females to stay on their natal territory for 20 replicates. (a2) and (b2) show the population average strategy of males to stay on their natal territory for 20 replicates. (a3) and (b3) show the population average strategies to produce a son for 20 replicates. (a) represents simulations where there was an equal probability for helpers and floaters to become a breeder, while (b) represents simulations where helpers had a higher probability of becoming a breeder than floaters.

393 probability between helpers and floaters (helpers having a higher chance of becoming breeders) the results
394 changed. In that case (Fig. 5B), females evolved to almost always stay, although in the lowest territory
395 qualities this probability decreased somewhat. Males also evolved to almost always stay, although in
396 multiple replicates a situation dependent strategy evolved where they left (probability to stay around 0%)
397 in very low quality territories and stayed (probability to stay around 100%) in high quality territories. Again,
398 a sex ratio strategy evolved where mostly daughters were produced in high quality territories and mostly
399 sons in low quality territories. We expect that this sex ratio strategy has evolved because it is beneficial for
400 high-quality territory parents to produce daughters, as these are the more efficient helpers, while it is likely
401 beneficial for low-quality territory parents to produce sons due to Fisher's principle.
402
403 **Discussion**
404 Here, we have shown that offspring sex-ratio strategies can evolve through multiple processes and that
405 these processes can cause similar sex ratio patterns. Therefore, we conclude that based solely on these
406 patterns it is impossible to disentangle the underlying processes. We showed that sex-ratio strategies can
407 evolve due to stochastic effects, due to a sex difference in philopatry and due to a sex difference in helping
408 efficiency. Moreover, we have shown that it can also depend on which traits co-evolved with the sex-ratio
409 strategies and what other factors influence these co-evolved traits. When there were benefits of
410 philopatry, the probability to remain philopatric evolved to high levels for both males and females,
411 compared to when there were no benefits of philopatry and a sex difference in staying probability evolved,
412 where females stayed while males left. Consequently, when a difference in staying probability evolved, this
413 then could be a cause for the evolution of the sex ratio strategies, while when no sex difference in staying
414 probability evolved a different mechanism had to have caused the evolution of sex ratio strategies.
415 Furthermore, we showed that individual differences in sex ratio strategies can evolve. When stochastic
416 processes drive part of the population in a certain direction, the other part will evolve to do the opposite
417 due to Fisher's principle, which can result in many different coexisting strategies.
418
419 Pen & Weissing (2000) made a similar, but analytical and simpler, model about the evolution of adaptive
420 quality-dependent offspring sex ratio strategies. They showed that on a population level, the offspring sex
421 ratio can deviate from 0.5 when the produced sex ratio depends on (territory) quality. They also included
422 a 'Seychelles warbler case study' and showed that in that case, they expected an overproduction of sons
423 on the population level. In our model we did not find any systematic biases in the population sex ratio. We
424 expect that this difference is caused by differences in underlying assumptions, and thus would like to point
425 out that small differences in underlying assumptions could lead to differences in results.
426
427 In our model, the probability to stay philopatric was fully determined by the chicks. This for example caused
428 that all the offspring stayed when there were benefits to philopatry. However, in empirical systems there
429 could be conflicts between parents and offspring on whether offspring stay, and parents might force their
430 offspring to leave the territory. This conflict could alter the evolutionary outcomes of whether offspring
431 stay or leave (Port et al., 2020), and in Seychelles warblers there are some clues that parents influence the
432 dispersal tendencies of their offspring (Borger et al., 2024).
433

434   One note of caution before applying our model to empirical research is that even though our model used
435   the Seychelles warbler case study, to this day no clear mechanism has been found by which birds could
436   manipulate their offspring sex ratio. In our model, such a mechanism is assumed and without it no
437   (adaptive or non-adaptive) sex ratio strategies can evolve. However, our model could be applied to
438   haplodiploid animals or to birds (and other chromosomal sex determining animal species) if a mechanism
439   does in fact exist. The evolved strategies in our model do correspond to the hypothesis posed by Komdeur
440   et al. (1997), and hence could explain the observed patterns if a sex ratio manipulation mechanism does
441   exist, yet we show that much less requirements are necessary for such a sex ratio trend to evolve (i.e. only
442   a difference in helping efficiency or in staying probability is necessary or even no difference at all).

444   The fact that a sex-difference in dispersal is important for the evolution of adaptive sex ratio strategies was
445   already mentioned by (Hamilton (1967). He described that when one sex disperses and the other remains
446   philopatric, animals should overproduce the dispersing sex, as this decreases kin competition. In our model,
447   we also found this (in the low quality territories), but also show that when multiple evolutionary forces
448   compete with it, the philopatric sex might be overproduced (e.g. in high quality territories). In empirical
449   systems it might be very common for all these adaptive forces to interplay and hence predicting what an
450   optimal sex ratio strategy should look like is a complex task that requires mathematical modelling or
451   simulations.

453   Moreover, using individual-based simulations to study evolutionary questions can gain new insights. Given
454   that it is relatively easy to increase the degrees of freedom in an individual-based model, it is a great tool
455   to study complex questions where evolutionary forces interact, or where traits co-evolve. Thus, we can
456   gain surprising results from individual-based simulations that without this tool might have otherwise not
457   been found.

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