

Heterospecific shoaling in an invasive poeciliid: shared history does not affect shoal cohesion

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1 **ABSTRACT**

2 Social behaviour potentially plays an important role in invasion success. New colonists, for example,
3 may glean useful information about predators and food by interacting with native heterospecifics.
4 The extent to which invaders benefit from such social interactions could hinge on their prior
5 exposure to other species. Here we ask how the shoaling decisions of the Trinidadian guppy, *Poecilia*
6 *reticulata*, - a successful invasive species - are mediated by their shared history with a heterospecific,
7 the phenotypically similar *Micropoecilia picta*. To do this, we monitored shoal cohesion in single-
8 species treatments and in treatments where *M. picta* was present. We predicted that shoal cohesion
9 would be greater in single than in mixed species shoals. We also hypothesised that mixed species
10 shoals consisting of fish with a shared history would be more cohesive than those where the two
11 species had hitherto occurred allopatrically. We found that shoal cohesion did not differ between
12 single and two-species treatments, or in relation to shared history with *M. picta*. However, we also
13 discovered that while guppies were more often found in mixed-species than single-species shoals,
14 they were more likely to have a conspecific individual as their nearest neighbour within mixed-
15 species shoals. These results show that guppies willingly shoal with heterospecifics, even in the
16 absence of a shared history, but also reveal that the resulting shoals are not randomly assembled.
17 This flexibility in shoaling may confer a crucial advantage in the initial stages of invasion.

18 **Keywords:** guppy, invasive species, *Poecilia reticulata*, shoal cohesion, shoaling, social behaviour

19 **INTRODUCTION**

20 Groups of fish that remain together for social reasons are defined as shoals (Kennedy & Pitcher,
21 1975; Pitcher, 1983) and it is in this context in which most social learning – the acquisition of skills
22 and knowledge from other individuals – occurs (Brown & Laland, 2003).

23 Joining a group confers many benefits, ranging from predator defence (Magurran, 1990; Stephens &
24 Sutherland, 1999) to improved food location (Pitcher, Magurran, & Winfield, 1982). It is not a

25 surprise, then, that social groups of fish are very common (Shaw, 1978). Shoaling with individuals
26 that are phenotypically and behaviourally similar is thought to be particularly effective for predator
27 defence due to the oddity effect (Landeau & Terborgh, 1986): individuals that stand out from the
28 group are more likely to be targeted by predators. Consequently, shoals tend to be assorted by size
29 (Croft et al., 2003; Krause & Godin, 1994; Ledesma & McRobert, 2008; Ward & Krause, 2001) and
30 colour (McRobert & Bradner, 1998; Rodgers, Kelley, & Morrell, 2010), in both conspecific and
31 heterospecific contexts (Crook, 1999; Krause, Godin, & Brown, 1996). There are, however,
32 accompanying costs, including increased parasite load (Barber, Hoare, & Krause, 2000) and reduced
33 mating opportunities (Griffiths, 1996). Fish therefore make the decision to associate with other
34 individuals by constant reappraisal of costs and benefits (Pitcher, 1983).

35 The guppy (*Poecilia reticulata*) has been introduced to every continent with the exception of
36 Antarctica, and is now established in at least 70 countries outside of its native range (Deacon,
37 Ramnarine, & Magurran, 2011). Social behaviour is thought to play an important role in invasion
38 success (Holway & Suarez, 1999) and, as a highly social species, this is may be especially true for the
39 guppy (Deacon & Magurran, 2016). Initial introductions are likely to be just a few individuals ,
40 possibly even a single pregnant female (Deacon et al., 2011). Shoaling benefits (e.g., dilution of
41 predation risk and improved foraging efficiency) may be especially critical at this stage of the
42 invasion process. If introduced fish also associate with heterospecifics, they further dilute the
43 predation risk. Any increase in survival and in population growth obtained as a result of social
44 interactions with invaders will also help mitigate Allee effects (Camacho-Cervantes, Garcia,
45 Ojanguren, & Magurran, 2014; Camacho-Cervantes, Ojanguren, Deacon, Ramnarine, & Magurran,
46 2014)

47 Prior experience can mediate shoaling decisions in two ways – through previous association such as
48 familiarity, and through shared evolutionary history. Familiarity is the tendency of individuals to
49 associate with those they have previously interacted with (Magurran, Seghers, Shaw, & Carvalho,

50 1994). Socially familiar shoals benefit from decreased resource competition (Höjesjö, Johnsson,
51 Petersson, & Järvi, 1998) and lower aggression due to the presence of more stable social hierarchies
52 (Johnsson, 1997). This may be due to lower perception of risk or elevated levels of social learning in
53 such assemblages (Morrell et al., 2008). Additionally, higher cohesion (more compactness) is
54 observed in shoals comprised of socially familiar individuals (Chivers, Brown, & Smith, 1995) and
55 produces more effective antipredator behaviour (Mathis & Smith, 1993).

56 Evolutionary history also shapes behaviour. For example, fish from localities where there are many
57 predators have a higher schooling tendency and stronger evasion behaviours than those from sites
58 where predation risk is reduced (Brown, Macnaughton, Elvidge, Ramnarine, & Godin, 2009;
59 Magurran & Seghers, 1990; O'Steen, Cullum, & Bennett, 2002; Seghers, 1973). Mate discrimination
60 is another behaviour that can be influenced by shared history. In Trinidad, for instance, the guppy is
61 found primarily in freshwater streams, though in certain locations its range extends into the habitat
62 of the phenotypically-similar species, the swamp guppy (*Micropoecilia picta*) which is usually
63 restricted to brackish water (Magurran & Ramnarine, 2004). Consequently, some guppy populations
64 occur sympatrically with *M. picta* (i.e. shared evolutionary history), while other populations do not.
65 Guppies that occur sympatrically with *M. picta* are less likely to attempt interspecific mating – even
66 if they have been reared in single species environments (Magurran & Ramnarine, 2004, 2005).

67 Here we take advantage of the natural experiment provided by the existence of allopatric and
68 sympatric populations of *P. reticulata* and *M. picta* in Trinidad to examine the shoaling decisions of
69 guppies. In the context of this work, the shared history of guppies that co-occur with *M. picta*
70 involves both evolutionary history and familiarity. Given the well documented advantages enjoyed
71 by conspecific shoals we predict that shoal cohesion will be greatest in single species shoals, as
72 opposed to shoals where two species are present irrespective of the origins of the heterospecific.
73 However, we also expect that if a heterospecific is present, shoal cohesion will be higher when the

74 fish are derived from populations with a shared history than those for which there has been no prior
75 contact.

76 **METHODS**

77 Experiments were conducted in July and August 2013 at The University of the West Indies.

78 *Fish collection & Ethical Note*

79 Female fish were collected using hand seines from the following locations in Trinidad: (A) Sumaria
80 Trace (guppies and *M. picta*) (B) Cunupia river (guppies and *M. picta*) (C) Caura river (guppies alone)
81 (D) Acono river (guppies alone) (E) Lower Tunapuna (guppies alone) (**Table 1**) and transported to the
82 laboratory in buckets filled with water from the location of capture at densities below 0.5g/L (40 fish
83 per 18L). As collection sites were within 20 minutes driving time from the university, we did not have
84 concerns about temperature or aeration levels causing stress to the fish. At the laboratory, they
85 were sorted by location and placed in single-species aquaria filled with aged tap water at maximum
86 densities of 50 individuals in a tank of dimensions 60 cm long x 30 cm wide x 30 cm high
87 (approximately 1 g per 5L). Fish were allowed time to acclimate to the laboratory conditions and
88 were housed in glass tanks lined with gravel and large stones, which made foraging easy and
89 minimised stress. The lab was illuminated between the hours of 7:00 am and 6:00 pm, in line with
90 the natural photoperiod. Fish were fed to satiation before and after experiments with TetraMin
91 flakes. Tanks were visually isolated from each other using sheets of white paper to limit stress.
92 Water quality was maintained by using sponge filters and performing partial water changes. Our
93 experiments did not involve any invasive sampling or any procedures that would cause pain to fish,
94 and handling times were minimised to limit stress. To further limit stress, tanks were also visually
95 isolated during behavioural trials and only exposed on one side to a single observer, who stayed as
96 still as possible. Fish were housed in the laboratory for no more than 6 weeks and upon completion
97 of experiments, were released into an ornamental pond on The University of the West Indies
98 campus. Guppies were used only once in experiments while some *M. picta* were reused due to low

99 numbers collected; upon emptying of the three *M. picta* stock tanks, the stock was replenished
100 randomly using fish from the 'used' fish tank. This encompassed the last third of the heterospecific
101 trials (approximately 100 *M. picta*). The probability of using the same three fish (see experiment
102 design below) in any two replicates was extremely low. Furthermore, all *M. picta* treatments had the
103 same number of trials that reused fish, so there was no consistent bias in reusing fish for any one
104 treatment.

105 *Experiments*

106 Six female fish were placed into a 90cm x 30 cm x 30 cm observation tank and allowed to settle for
107 10 minutes. Females were chosen because they have stronger shoaling tendencies than males
108 (Magurran, 2005). Shoals contained a range of sizes, and the size of the focal fish was recorded as
109 small (17-21mm TL), medium (22-26mm) or large (27-31mm). *M. picta* females were chosen to be in
110 the same size-range as the female guppies. Depending on the treatment, replicates included two
111 sets of 3 guppies, or 3 guppies and 3 *M. picta*, from either the same or different populations. In pilot
112 trials, 10 minutes was found to be the time required for fish to settle and swim normally. This was
113 then used as the time for settling in experimental trials.

114 One focal guppy was chosen and the species of, and distance to, the nearest neighbour (in guppy
115 body lengths, approximately 3cm) was recorded every 20 seconds for 10 minutes by one of two
116 observers, who viewed the experimental tank from the side. Given that fish change position
117 frequently within a shoal (Krause, 1993; Partridge, 1982; Pitcher, 1973), we believe that the distance
118 from a single focal fish to the nearest neighbour, when measured with high replication and repeated
119 within-trial measurements, captures shoal conformation. Furthermore, the nearest neighbour
120 distance can be accurately scored by eye (Pitcher & Parrish, 1993) and is unlikely to be affected by
121 fish size as our experiments randomised fish size and thus there would be no systematic bias or
122 correlation between fish size and treatment. The presence of mixed shoals, i.e. at least three
123 individuals of different species within 4 body lengths of one another (Pitcher & Parrish, 1993) was

124 also recorded along with each distance measurement (every 20 seconds). This was repeated for 20
125 females from each of 5 guppy populations.

126 Treatments were designed to allow for contrasts between conspecific and heterospecific shoals as
127 well as between heterospecific shoals in which guppies either did or did not possess a shared history
128 with *M. picta* (**Table 2**), while avoiding the introduction of site as a confounding factor. For example,
129 when comparing the response of guppies from site C to guppies from site A or *M. picta* from site A,
130 we did not need to be concerned about the origin of the second three fish affecting our
131 interpretation.

132 *Statistical methods*

133 For each focal guppy in a mixed species trial, the remaining 5 fish consisted of 2 guppies and 3 *M.*
134 *picta*. Therefore, if shoaling randomly, the focal fish's nearest neighbour should be a guppy 40% of
135 the time. The observed values for all mixed species replicates (20 replicates per treatment for 5
136 treatments, see Table A1, Supplementary Material) were compared with this expected proportion
137 using a one sample t-test.

138 Initial investigation of distance data revealed a Poisson-type distribution with a strong positive skew
139 (**Fig. 1**). Simple and generalised linear models with appropriate error structure were unable to
140 provide a good fit to the data. Mixed-effects and generalised mixed-effects models provided better
141 model diagnostics but suffered from convergence problems. A Bayesian generalised linear mixed-
142 effects modelling approach was therefore used instead, via the R package MCMCglmm (Hadfield,
143 2010). We used a weakly informative inverse-gamma prior and ran models for 1 000 000 iterations
144 with a burn-in of 50 000 and a thinning interval of 100. Separate models were used to examine the
145 effect of the presence of *M. picta* and shared history with *M. picta* on shoal cohesion. Other
146 variables included in both models were observer and focal fish size. A random effect for focal fish
147 identity was included to account for pseudo-replication and over-dispersion in the data. Variation in
148 distance estimates between two observers was accounted for in the model. Density plots and 95%

149 highest posterior density intervals (which are akin to 95% confidence intervals) were used to
150 interpret significance of terms (Hadfield, 2015). All analyses were carried out using the statistics
151 software, R (R Development Core Team, 2016).

152 **RESULTS**

153 A one sample t-test of combined data for all sites revealed that guppies had a conspecific neighbour
154 significantly more often than expected in mixed species treatments ($t_{99} = 9.798$, $P < 0.001$; data in
155 Table A1, Supplementary Material). This relationship was the same for all five sites (Site A: $t_{19} =$
156 5.293 , $P < 0.0001$; Site B: $t_{19} = 3.434$, $P < 0.01$; Site C: $t_{19} = 6.717$, $P < 0.00001$ $2.026e-06$; Site D: $t_{19} =$
157 3.971 , $P < 0.001$; Site E: $t_{19} = 3.760$, $P < 0.01$). More than 80% of mixed species trials consisted of
158 more than 80% mixed shoals.

159 MCMCglmm models indicate that neither the presence of a second species, nor shared history, had a
160 significant effect on the nearest neighbour distance. Density plots (**Figs. A1 & A2**, Appendix) and 95%
161 highest posterior density intervals (**Table 3** and **Table 4**) show that the effect of a second species and
162 shared history lie around zero suggesting that these variables are non-significant. Trace plots (**Figs.**
163 **A1 & A2**, Appendix) show no obvious trends, suggesting that the model ran successfully, and
164 autocorrelation values were all below 0.05. There was a significant observer effect, but our model
165 accounts for this and preserves our interpretation of the data.

166 **DISCUSSION**

167 Our findings show that guppies freely shoal with a phenotypically similar heterospecific and that
168 shoal cohesion is unaffected by the presence of this species, whether or not guppies come from
169 populations that have a shared history with the heterospecific. This consistency in shoaling
170 behaviour in the face of novel social conditions could be an important factor in guppy invasive
171 success.

172 *Mixed shoals*

173 Our results support previous findings that guppies will readily form shoals with *M. picta* (Camacho-
174 Cervantes, Ojanguren, et al., 2014). That is, guppies are more likely to be found in mixed shoals
175 when the alternative is either to be alone or part of a smaller single-species shoal. Earlier work with
176 these species entailed choice experiments, where shoals were held in clear bottles and the time
177 spent with each shoal used to quantify preference (Camacho-Cervantes, Ojanguren, et al., 2014). By
178 observing the focal fish in a free-swimming shoal, our design allowed us to quantify shoal
179 compactness, something not possible with a barrier between the focal fish and other individuals.
180 This enabled us to observe the structuring within a shoal, as well as the focal fish's readiness to shoal
181 with heterospecifics. By better replicating a natural situation, our results add a level of ecological
182 validity to the previous work on these two species. Indeed, we found that guppies more frequently
183 had a conspecific than heterospecific nearest neighbour.

184 *Shared history*

185 Additionally, we find that guppies shoal just as closely with a heterospecific as with a conspecific,
186 whether or not they possess shared history. This is surprising given that social familiarity and genetic
187 relatedness are known rules of guppy shoal assembly (Barbosa, Camacho-Cervantes, & Ojanguren,
188 2016) and one would expect that shoal cohesion might decrease in the presence of an unfamiliar
189 species. Thus, contrary to expectations, guppies behave as 'friendly' shoal-mates towards *M. picta*,
190 despite evolutionary naivety.

191 *The formation of a sub-shoal*

192 Our results enhance understanding of how shoals are organised in mixed species contexts: Guppies
193 often form mixed shoals where the nearest fish is more likely to be a conspecific (**Fig. 2**), effectively
194 producing a guppy 'sub-shoal' within a heterospecific shoal. This conformation does not appear to
195 depend on shared history. It is possible that guppies may be unable to avoid contact if *M. picta* was
196 shoaling very closely. However, comparison to the guppy-only control suggests that either *M. picta*

197 individuals shoal in as close proximity to guppies as guppies do to one another, or that guppies make
198 no distinction between conspecifics and *M. picta*.

199

200

201 *Explanation and implications*

202 Guppies are known to use visual and olfactory cues in identifying conspecifics (Magurran & Seghers,
203 1994; Morrell et al., 2008) and can judge their phenotype relative to others (Gasparini, Serena, &
204 Pilastro, 2013; Řežucha & Reichard, 2015). Also, phenotype and social familiarity are both factors
205 influencing decisions to shoal (Barbosa et al., 2016). Consequently, identification of individuals from
206 different populations, or of different species, should not be difficult. Yet males may still attempt to
207 mate with female *M. picta* (Liley, 1966) and in this study females associate with *M. picta* as closely as
208 they would with other guppies.

209 There are two potential explanations for this. Firstly, it is possible that guppies cannot distinguish
210 themselves from *M. picta*. This is unlikely given that it has been established that visual and chemical
211 cues are important, and may even be so finely tuned that they vary between populations (Brown,
212 Elvidge, Macnaughton, Ramnarine, & Godin, 2010). The alternative explanation is that while they are
213 able to distinguish between *M. picta* and conspecifics, a choice is made to shoal with heterospecifics
214 (either by actively seeking them out, or by choosing not to avoid them) as they are sufficiently
215 phenotypically similar and pose little threat. Investigating whether there is indeed a distinct
216 phenotypic or chemical threshold beyond which shoaling behaviour changes may be an interesting
217 area for future work.

218 Previous work has shown that learning is important in male guppy mate discrimination between
219 conspecific and *M. picta* females (Haskins & Haskins, 1949; Liley, 1966; Magurran & Ramnarine,
220 2004). If learning in heterospecific contexts is important in mating decisions, it may also be

221 important for heterospecific shoaling decisions. However, guppies that had a shared history and thus
222 the opportunity to learn to discriminate *M. picta* showed no difference in shoal cohesion to guppies
223 that were not previously exposed. This suggests that, even when given the opportunity to learn
224 discrimination between conspecifics and *M. picta*, guppies still make the choice to shoal with this
225 heterospecific.

226 Notwithstanding our findings, preference for a single species vs mixed species shoal cannot be
227 discerned as the present experiment did not offer a direct choice. It is possible that the formation of
228 mixed shoals was due to *M. picta* behaviour, rather than that of the guppies. This would be an
229 interesting line for future investigation - at what shoal size or at what conspecific-heterospecific
230 ratio might single-species shoals be more common? This would shed light on the process of decision
231 making in a social landscape. Even more illuminating will be whether costs and benefits (foraging,
232 parasite load etc.) can be shown to validate these decision thresholds. Further investigation using
233 inter-individual distances and tracking for an entire shoal (e.g. Tang, Wu, Huang, Kuang, & Fu, 2017)
234 may reveal more complicated heterospecific shoal dynamics.

235 Fish were allowed time to settle into the laboratory setting and the observational tank before trials
236 began, and these two species are commonly found in mixed species shoals in certain Trinidadian
237 streams (Magurran & Ramnarine, 2004) therefore we can assume that these findings are relevant to
238 the wild. We do not believe that size-assortative shoaling (Krause et al., 1996) affected our results as
239 each experimental shoal comprised a range of sizes and the size of the focal fish was not significant
240 in our model. Furthermore, while average guppy and *M. picta* size may differ (Torres-Dowdall,
241 Dargent, Handelsman, Ramnarine, & Ghalambor, 2013) females show indeterminate growth
242 (Magurran, 2005) and these species show a broad overlap in size.

243 *Conclusion*

244 Overall, our findings support the idea that the behaviour of this gregarious invasive species is
245 unaltered by the presence of a heterospecific and that shoaling behaviour is likely a substantive

246 contributor to the invasive success of guppies. Social learning will presumably be most effective for a
247 species that associates closely with individuals of the other species. Thus, guppies in a new
248 environment can potentially be very effective at exploiting social information (sensu Brown &
249 Laland, 2003) and avoiding Allee effects. Understanding the characteristics that predispose a
250 species to be invasive will require examination of the social and behavioural mechanisms that
251 function in the very early stages of invasion.

252

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258

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420 **Tables (including legends/headings)**

421

422 **Table 1:** Location of field sites where fish were collected

Site	Latitude	Longitude	Guppies present?	<i>M. picta</i> present?
A Sumaria Trace	10.56424	-61.43031	✓	✓
B Cunupia River	10.56156	-61.41769	✓	✓
C Caura River	10.68877	-61.35829	✓	X
D Acono River	10.708	-61.40001	✓	X
E Tunapuna River	10.62496	-61.40709	✓	X

423

424

425 **Table 2:** Number of replicates for each treatment, in the context of the experimental design.

Hypothesis		Site		Number of replicates
1: Species context	2: Shared history	1 st 3 fish	2 nd 3 fish	
Conspecific shoals	Not applicable to conspecific shoals	A	A	20
		B	B	20
		C	A	20
		D	B	20
		E	B	20
Heterospecific shoals	Shoals with shared history	A	A	20
		B	B	20
	Shoals without shared history	C	A	20
		D	B	20
		E	B	20

426 The first three fish were always guppies and the second three were either guppies or *M. picta* depending on

427 whether it was a conspecific or heterospecific treatment. Hypotheses: (1) guppies form more compact shoals

428 in conspecific than heterospecific treatments and (2) shoals are least compact in heterospecific treatments

429 where they are naïve to *M. picta*. See Table 1 for key to sites.

430

431

432

433 **Table 3** Results of a Markov Chain Monte Carlo generalised linear mixed effects model including presence of a
434 second species.

	post.mean	l-95% CI	u-95% CI
Intercept	0.350	0.205	0.490
Species 2	0.094	-0.028	0.225
Observer 2	0.181	0.050	0.305
Focal Fish Size - Large	-0.045	-0.193	0.110
Focal Fish Size - Small	0.133	-0.029	0.290

435

436 Posterior mean (post.mean) and lower and upper 95% posterior density estimates (l-95% CI and u-95% CI,
437 respectively) from a Markov Chain Monte Carlo generalised linear mixed effects model. (Species 2- presence of
438 a second species).

439 **Table 4** Results of a Markov Chain Monte Carlo generalised linear mixed effects model including shared
440 history.

	post.mean	l-95% CI	u-95% CI
Intercept	0.346	0.205	0.493
Shared history	0.143	-0.031	0.309
No shared history	0.062	-0.085	0.209
Observer 2	0.179	0.0479	0.305
Focal Fish Size - Large	-0.039	-0.191	0.109
Focal Fish Size Small	0.137	-0.018	0.300

441

442 Posterior mean (post.mean) and lower and upper 95% posterior density estimates (l-95% CI and u-95% CI,
443 respectively) from a Markov Chain Monte Carlo generalised linear mixed effects model. (SEH-shared
444 evolutionary history).

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448

450 **Table A1:** The frequency at which a guppy was found to be the nearest neighbour for all
 451 mixed species replicates. G=Guppy, P= *M. Picta*; A-E=sites

Treatment	Replicate	Number of times a guppy is the nearest neighbour	Number of times <i>M. picta</i> is the nearest neighbour	Percent of the time a guppy is the nearest neighbour
GAPA	1	19	11	63.33
	2	17	13	56.67
	3	10	20	33.33
	4	18	12	60.00
	5	16	15	53.33
	6	15	14	50.00
	7	18	12	60.00
	8	18	12	60.00
	9	13	17	43.33
	10	13	17	43.33
	11	17	13	56.67
	12	17	13	56.67
	13	18	12	60.00
	14	11	19	36.67
	15	12	18	40.00
	16	15	15	50.00
	17	19	11	63.33
	18	17	13	56.67
	19	18	12	60.00
	GBPB	20	10	20
1		17	13	56.67
2		18	12	60.00
3		18	12	60.00
4		18	12	60.00
5		7	23	23.33
6		11	19	36.67
7		15	15	50.00
8		17	13	56.67
9		17	13	56.67
10		11	19	36.67
11		13	17	43.33
12		16	14	53.33
13		17	13	56.67
14		21	9	70.00
15		16	14	53.33
16		14	16	46.67
17	18	12	60.00	

	18	12	18	40.00
	19	6	24	20.00
	20	18	12	60.00
GCPA	1	21	9	70.00
	2	23	7	76.67
	3	26	4	86.67
	4	14	16	46.67
	5	16	14	53.33
	6	9	21	30.00
	7	19	11	63.33
	8	23	7	76.67
	9	18	12	60.00
	10	16	14	53.33
	11	21	9	70.00
	12	21	9	70.00
	13	20	10	66.67
	14	21	9	70.00
	15	18	12	60.00
	16	16	14	53.33
	17	16	14	53.33
	18	17	13	56.67
	19	17	13	56.67
	20	11	19	36.67
GDPB	1	11	19	36.67
	2	14	16	46.67
	3	19	11	63.33
	4	18	12	60.00
	5	14	16	46.67
	6	12	18	40.00
	7	16	14	53.33
	8	18	12	60.00
	9	3	27	10.00
	10	19	11	63.33
	11	12	18	40.00
	12	22	8	73.33
	13	19	11	63.33
	14	20	10	66.67
	15	14	16	46.67
	16	20	10	66.67
	17	23	7	76.67
	18	12	18	40.00
	19	20	10	66.67
	20	18	12	60.00
GEPB	1	10	20	33.33
	2	20	10	66.67
	3	19	11	63.33
	4	17	13	56.67

5	11	19	36.67
6	25	5	83.33
7	19	11	63.33
8	18	12	60.00
9	9	21	30.00
10	23	7	76.67
11	30	0	100.00
12	11	19	36.67
13	19	11	63.33
14	14	16	46.67
15	18	12	60.00
16	9	21	30.00
17	18	12	60.00
18	9	21	30.00
19	18	12	60.00
20	19	11	63.33

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453

454 **Figure Legends (Main text)**

455 **Figure 1:** Frequency distribution of nearest neighbour distances for all trials combined. (Distance to nearest
456 fish measured in guppy body lengths, ~ 3cm)

457 **Figure 2:** A pictorial representation of our results, showing the shoaling behaviour of guppies (blue) and *M.*
458 *picta* (red). The symbols ✓ and X describe whether guppies have (✓) or do not have (X) shared evolutionary
459 history with *M. picta*. Conformation A (nearest neighbour is a guppy) occurs more frequently than either B or C
460 (nearest neighbour is *M. picta*). The distances to the nearest fish (a, b and c) do not differ between
461 conformations. (to be reproduced in colour)

462

463 **Appendix**

464

465 **Figure A1:** Density curves (left) and trace plots (right) for parameter estimates from a
466 Markov Chain Monte Carlo generalised mixed effects model of shoal cohesion: (a) intercept,

467 (b) presence of a second species, (c) observer effect, (d) focal fish size (large) and (e) focal
468 fish size (small). Left panel: x-axis: parameter estimate, y-axis: relative density; Right panel:
469 x-axis: number of iterations ($\times 10^3$), y-axis: parameter estimate.

470

471 **Figure A2:** Density curves (left) and trace plots (right) for parameter estimates from a
472 Markov Chain Monte Carlo generalised mixed effects model of shoal cohesion: (a) intercept,
473 (b) shared evolutionary history, (c) no shared evolutionary history, (d) observer effect, (e)
474 focal fish size (large) and (f) focal fish size (small). Left panel: x-axis: parameter estimate, y-
475 axis: relative density; Right panel: x-axis: number of iterations ($\times 10^3$), y-axis: parameter
476 estimate.

477

478

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Figure 1
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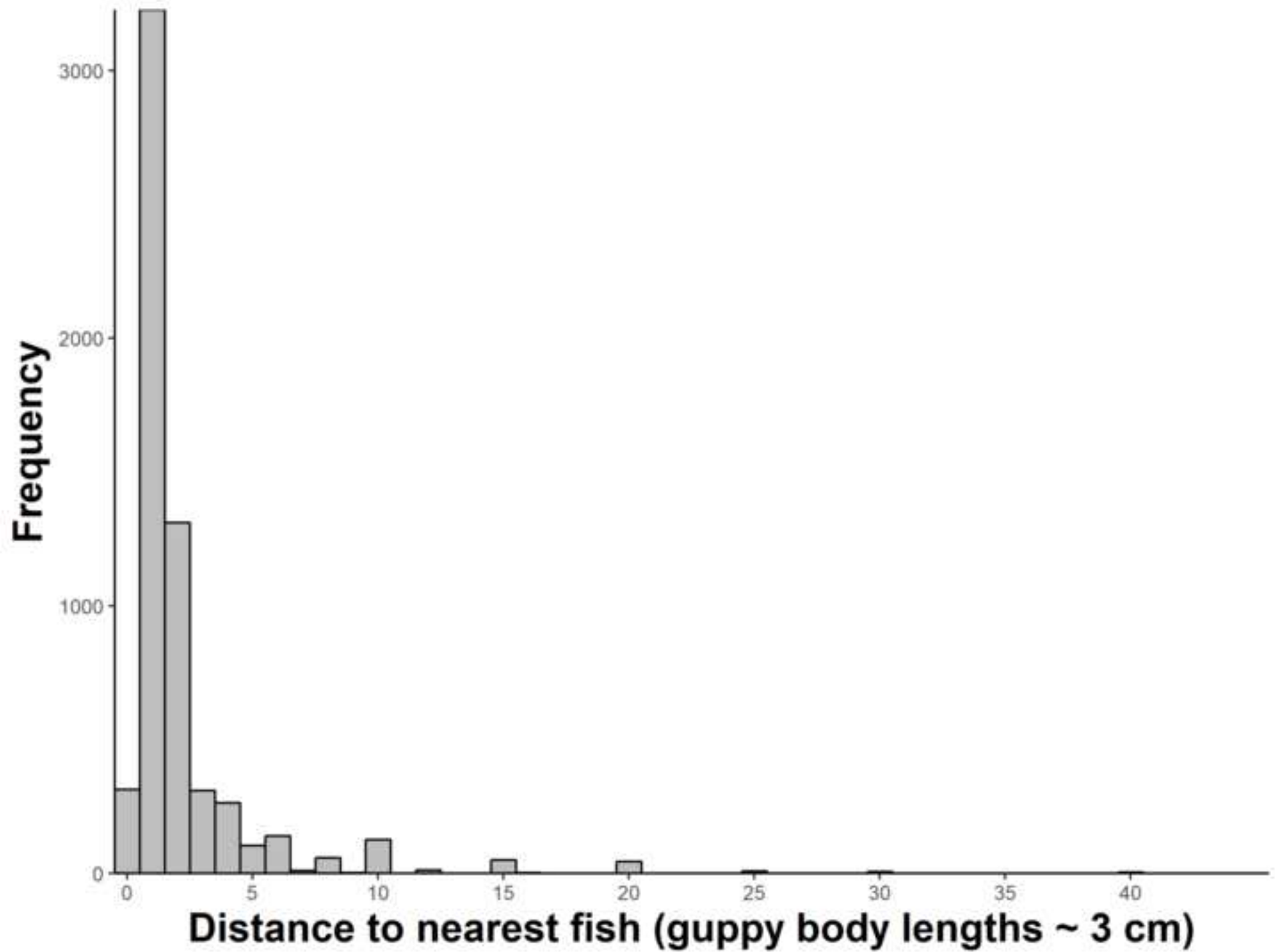


Figure 2
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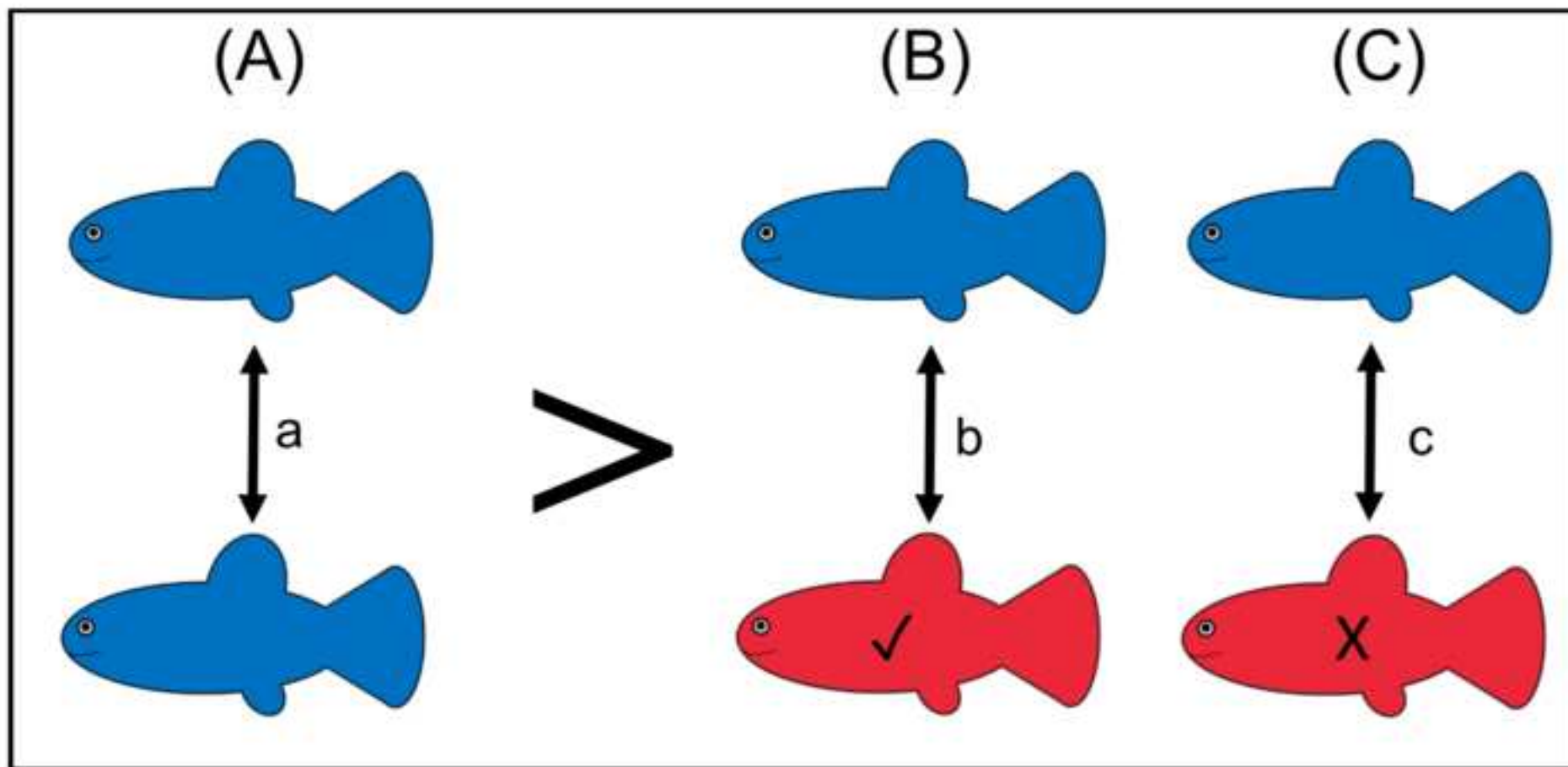


Figure A1
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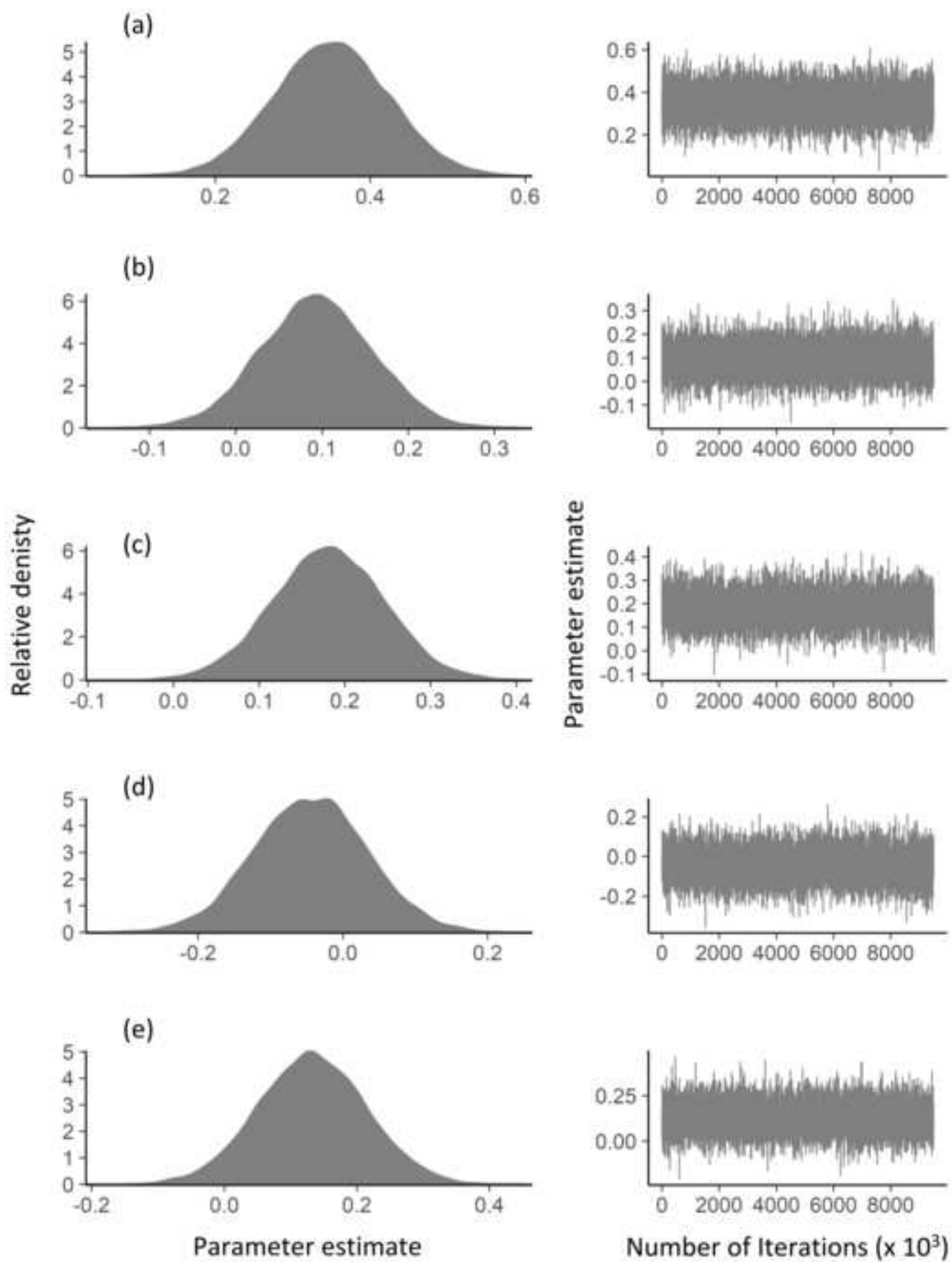


Figure A2
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