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# Spatially clustered resources increase male aggregation and mating duration in *Drosophila melanogaster*

Churchill, ER

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2 and mating duration in *Drosophila melanogaster*

3

4 ABSTRACT

5 In environments where females mate multiply, males should adjust their behaviour and  
6 physiology in response to the perceived level of sperm competition in order to  
7 maximise their fitness. Evidence of such plasticity has been found in a number of  
8 laboratory and field studies, but little is yet known about the cues stimulating these  
9 responses in natural populations. One way in which males appear to assess sperm  
10 competition risk is through encounter rates with conspecific males. Such encounter  
11 rates may be driven by the spatial distribution of resources required by males (i.e. food  
12 patches or potential mates), which in turn affects local density. However, explicit links  
13 between resource distribution, male encounter rate, and shifts in behaviour related to  
14 sperm competition have not been demonstrated. We show that when group size of *D.*  
15 *melanogaster* males is held constant, a small decrease in the distance between  
16 patches of food resources has striking effects on male behaviour. First, males on  
17 clustered resources have a significantly reduced inter-male distance (and hence  
18 encounter rate) compared to those on dispersed resources, and second, males from  
19 clustered resources show an increase in subsequent non-competitive copulation  
20 duration – previously shown to be a reliable indicator of male perception of sperm  
21 competition risk – of more than two minutes (13%) compared to those from dispersed  
22 resources. The aggregation of resources, operating via increased encounter rate, can  
23 stimulate shifts in behaviour affecting male sperm competition performance. Given that  
24 the spatial distribution of resources, is typically variable in natural populations (and  
25 often unpredictable), selection is likely to favour the evolution of plasticity in sexual  
26 behaviour where resource aggregation increases the probability of sperm competition.

27 Keywords

28 Copulation duration, evolution, mating behaviour, plasticity, resource distribution,

29 sexual conflict, sexual selection, sperm competition

30 Introduction

31 Variation in population density affects the rate at which individuals encounter  
32 conspecific competitors and potential mates, with consequences for the strength of  
33 sexual selection. One source of variation in local population density is the spatial  
34 distribution of critical resources. Clumped resources lead to increased encounter rates  
35 with competitors and mates as they gather to access those resources (Emlen & Oring,  
36 1977). Where encounter rate is high, investment in traits such as sperm production,  
37 courtship, mating duration should be upregulated to maximise reproductive success  
38 in a dense social environment (Kokko & Rankin, 2006). Several empirical studies have  
39 supported this prediction, including in crickets (Gage & Barnard, 1996), beetles  
40 (McCullough, Buzatto, & Simmons, 2018), bugs (García-González & Gomendio,  
41 2004), platyhelminths (Giannakara, Schärer, & Ramm, 2016), fish (Candolin &  
42 Reynolds, 2002), and rodents (Firman, Garcia-Gonzalez, Simmons, & André, 2018;  
43 Ramm & Stockley, 2009).

44 Demonstrating that male encounter rate can stimulate plasticity in sexual traits has  
45 generally been achieved by housing males at varying densities in the laboratory, with  
46 the most common treatment comparing a singly-housed male with a male housed with  
47 one or more conspecifics (Candolin & Reynolds, 2002; Firman et al., 2018; Gage &  
48 Barnard, 1996; Lizé et al., 2012; Moatt, Dytham, & Thom, 2013). This extreme  
49 manipulation of the total number of potential rivals is not intended to mimic the effects  
50 males experience in nature, but rather to demonstrate that such adaptive responses  
51 exist. Evidence for how such responses link to more ecologically-realistic stimuli is  
52 therefore lacking, although effects of sperm competition have been observed in natural  
53 populations – for example in lizards (Kustra, Kahrl, Reedy, Warner, & Cox, 2019) and  
54 frogs (Buzatto, Roberts, & Simmons, 2015). Given that patchiness in food resources

55 is common in nature, and that resource distribution affects the degree of male-male  
56 competition (Emlen & Oring, 1977), small-scale variation in resource distribution that  
57 leads to local variation in encounter rate should drive plastic variation in the allocation  
58 of resources by males to sexual behaviour described above.

59 Laboratory studies have repeatedly demonstrated that *Drosophila melanogaster*  
60 (*Drosophilidae Diptera*) males are highly sensitive to the presence of other males, and  
61 that they increase their investment in sperm quality and ejaculate size (Garbaczewska,  
62 Billeter, & Levine, 2013; Hopkins et al., 2019; Moatt, Dytham, & Thom, 2014),  
63 investment in ejaculate composition (Fedorka, Winterhalter, & Ware, 2011; Hopkins et  
64 al., 2019; Wigby et al., 2009), and lengthen copulation durations (Bretman, Fricke, &  
65 Chapman, 2009) when they perceive an elevated risk of sperm competition. Because  
66 *D. melanogaster* feed and breed on fermenting fruit (Begon, 1982), they rely on an  
67 inherently patchy resource with individual fruits naturally varying in size and proximity.  
68 Sex ratio and local population density of natural populations can vary considerably as  
69 a result (Markow, 1988; Soto-Yéber, Soto-Ortiz, Godoy, & Godoy-Herrera, 2018).  
70 Such patchiness in natural food resources seems an ideal candidate for the type of  
71 ecological variability that might stimulate adjustment in post-copulatory processes in  
72 the wild.

73 In this study, we test whether sperm competition-linked responses respond to resource  
74 patchiness by exposing male *D. melanogaster* to three different food distributions  
75 (clustered, dispersed and a uniform coverage control). In this way we can manipulate  
76 local density in an ecologically-realistic way, but without manipulating the number of  
77 rivals as previous laboratory studies have done (Bretman et al., 2009; Fedorka et al.,  
78 2011; Garbaczewska et al., 2013; Hopkins et al., 2019; Moatt et al., 2014; Wigby et  
79 al., 2009). We use the duration of copulation as a proxy for males' perception of sperm

80 competition risk, an association that has been demonstrated repeatedly in the  
81 laboratory (Bretman et al., 2009; Bretman, Fricke, Hetherington, Stone, & Chapman,  
82 2010; Bretman, Westmancoat James, Gage Matthew, & Chapman, 2012; Bretman,  
83 Westmancoat, & Chapman, 2013; Mazzi, Kesäniemi, Hoikkala, & Klappert, 2009;  
84 Moatt et al., 2013). We predict that: (a) by experimentally manipulating the distribution  
85 of food resources, males on clustered resources have a higher mean proximity to rivals  
86 (i.e. a higher encounter rate on average), and (b) males on clustered resources will  
87 subsequently mate for longer on average, indicating an adaptive response based on  
88 perception of increased sperm competition risk.

89

90 Methods

91 All fly rearing and experiments were conducted in a 12 hour light:dark cycle (0800 –  
92 2000 GMT), at 25 °C. *Drosophila melanogaster* used were from a laboratory  
93 population (Canton-S), and populations were cultured on 7 ml of a standard agar-  
94 based medium of 40 g of yeast per litre, in 40 ml vials. Between 20 and 30 *Drosophila*  
95 were housed in each vial. To minimise any effects of inbreeding, drift, and selective  
96 sweeps, every seven days the adults from all vials were pooled and randomly  
97 redistributed among new vials to start the next generation.

98 Test flies (180 in total – 60 per treatment) were collected from parent vials, each  
99 established with six males and six females allowed to breed for 70-98 h. Test flies  
100 were removed from parent vials within six hours of eclosion to ensure virginity; prior to  
101 this individuals are not sexually mature (Strömnæs & Kvelland, 1962). Flies were  
102 immediately aspirated under light ice anaesthesia into treatments. Virgin female flies  
103 for mating assays were collected from the same parental vials and aspirated into new  
104 vials in groups of four. Females were used in mating assays when they were seven  
105 days (+ 6-8 hours) old (Churchill, Dytham, & Thom, 2019).

106 Manipulating resource distributions and patchiness

107 Each replicate for each treatment consisted of four virgin males maintained in a 90  
108 mm Petri dish for three days. Food in each of these 45 dishes was arranged in one of  
109 three treatments ( $N = 15$ ): clustered, dispersed or uniform food resource distributions.  
110 Clustered and dispersed treatments both contained four plugs ( $420 \text{ mm}^3$  per patch) of  
111 standard food medium (as described above). The size of these patches is within the  
112 range of patch sizes where territorial behaviours have previously been observed  
113 (Hoffmann & Cacoyianni, 1990).

114 Dispersed food discs were placed at four equidistant points around the circumference  
115 of the Petri dish; these were 50 mm apart along the edge of the square, 70 mm apart  
116 on the diagonal (illustrated in Fig. 2). Clustered discs were placed in the centre of the  
117 Petri dish, in a square arrangement with each food disc in direct contact with adjacent  
118 discs. The uniform treatment was an even layer of 45 ml standard medium covering  
119 the bottom of the dish (to the same height as the four food patches in the previous two  
120 treatments): volume and surface area were both greater in the uniform than the two  
121 patchy treatments, but given the number of flies food was assumed to be available *ad*  
122 *libitum* in all. All treatments were maintained in 12L:12D at 25 °C, and the four male  
123 flies per treatment remained in these conditions for 70 hours (+/- 1 h) until aged to  
124 three days.

125

#### 126 Quantifying male spacing behaviour

127 Treatment enclosures were placed in one of two identical incubators maintained at 25  
128 °C and on the same 12:12 L:D cycle as the stock flies. Each incubator was fitted with  
129 a Raspberry Pi ([www.raspberrypi.org](http://www.raspberrypi.org)) connected to an 8MP Raspberry Pi Camera  
130 module (v2; [www.thepihut.com](http://www.thepihut.com)). Two to three Petri dishes, placed in a balanced  
131 arrangement across all treatment combinations, were placed directly under each  
132 camera. We used frame capture software ('raspistill') to collect one image every 15  
133 minutes from 0800-2000 GMT (during the light part of the cycle). We captured the x-y  
134 coordinates of each male at each time point using ImageJ's multiple point selector tool  
135 (Schneider, Rasband, & Eliceiri, 2012), and then converted these into a set of six  
136 Euclidean pairwise distances between the four males (24670 measurements across  
137 the three treatments and all time points). For 325 out of the 4290 individual time-point  
138 photographs (7.6%) we were unable to accurately locate at least one male on the

139 image. To minimize the effect of missing data on the number of time points included  
140 per replicate, the unit of analysis was the mean (rather than the raw data) of the  
141 distances between each pair for each time point.

142

#### 143 Reproductive behavioural assays

144 After 70 h in treatment, each male from each Petri dish was allowed one opportunity  
145 to mate with a virgin female and mating behaviours were observed ( $N = 15$ ; 60  
146 individuals). The male and female were aspirated into a standard food vial  
147 supplemented with  $\sim 0.03$  g active yeast granules. The space in the vial was limited to  
148  $7\text{cm}^3$  by pushing the vial bung down into the vial to reduce encounter latency.

149 Courtship latency was defined as the time from which the pair were first introduced  
150 until the male initiated his first wing extension. Latency to copulate (courtship duration)  
151 started at the time of the first wing extension, and ended with a male's successful  
152 mounting attempt. Copulation duration was recorded from successful mounting until  
153 the pair were fully separated.

154 Not every male courted (uniform: 81.8%; clustered: 86.4%; dispersed: 95.6%), and not  
155 all courting males mated (uniform: 75.0%; clustered: 86.8%; dispersed: 83.3%). We  
156 observed each pair for a maximum of 90 minutes after the pair had been introduced,  
157 and recorded failure to court and/or failure to mate after this time.

158

#### 159 Statistical analysis

160 Sample sizes were 15 replicates ( $N = 60$  *Drosophila*) for each of the three treatments,  
161 of which 11 from each treatment (33 in total) were photographed to collect spacing  
162 data. The effect of treatment on total inter-male distance was analysed using linear

163 mixed effects models, with plate included as a random effect in all models to account  
164 for the non-independence of the four males in a single treatment replicate. Time point  
165 (numbered sequentially from first to last measurement and treated as continuous) was  
166 modelled as a fixed effect.

167 Treatment effects on mating related traits were analysed using linear mixed effects  
168 models, with replicate plate entered as a random effect to account for the fact that  
169 mating data were available for (up to) four males per plate. Time point) and treatment  
170 were initially entered as interacting predictor variables; if the interaction was non-  
171 significant we re-ran the model with both variables entered as main effects. We used  
172 the R package lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017) to generate p  
173 values using the Satterthwaite approximation for degrees of freedom. To assess the  
174 effect of treatment on binomial variables (courtship success, copulation success) we  
175 used generalised linear mixed models with a binomial error distribution, and replicate  
176 plate nested within treatment to account for possible plate effects.

177

178 Animal welfare note

179 Although *Drosophila* are not currently subject to any ethical restrictions in the United  
180 Kingdom, we took precautions to minimise injury and stress by controlling larval  
181 density during development, handling flies minimally and using only light ice  
182 anaesthesia, and by euthanizing flies at the end of the experiment while they were  
183 under anaesthesia.

184

185

186 Results

187 Effect of food distribution on inter-male spacing

188 The spatial distribution of food patches significantly influenced the mean pairwise  
189 distance between the four males in the treatment, and this interacted with the time  
190 course of exposure to treatment (treatment\*time:  $F_{2,4239} = 286$ ,  $P = 2.20e^{-11}$ ; Fig. 1;  
191 Table 1). On the final day of treatment the time effect had stabilized (treatment\*time  
192  $F_{2,525} = 1.134$ ,  $P = 0.3224$ ), leaving a significant main effect of treatment on pairwise  
193 distance between males ( $F_{2,30} = 32.268$ ,  $P = 3.33e^{-8}$ ; interaction removed; Table 1).  
194 Post-hoc testing confirmed that on this final day, pairwise distances among males in  
195 the dispersed treatment ( $44.02 \pm 0.66$  mm SE) and the uniform treatment ( $39.35 \pm$   
196  $0.93$  mm SE) were both significantly greater than among males in the clustered food  
197 treatment ( $22.79 \pm 0.86$  mm SE; dispersed vs clustered  $F_{1,20} = 57.8$ ,  $P = 2.53e^{-7}$ ;  
198 uniform vs clustered:  $F_{1,20} = 27.9$ ,  $P = 3.63e^{-5}$ ; time remained in these models as a  
199 main effect). There was no significant difference in mean pairwise distance between  
200 males in the uniform and dispersed treatments ( $F_{1,20} = 3.9$ ,  $P = 0.061$ ).

201

202 Effect of food distribution on mating behaviour

203 Among those males that mated, copulation duration was significantly affected by food  
204 distribution previously experienced by males ( $F_{2,42.5} = 3.96$ ,  $P = 0.026$ ; Fig. 2).  
205 Analysing the effect of treatment on the mean mating duration across all males in a  
206 replicate – a more conservative measure – confirmed a significant difference in mating  
207 durations between treatments ( $F_{2,42} = 4.22$ ,  $P = 0.021$ ). Males from the clustered  
208 treatment mated for significantly longer ( $1170 \pm 28$  s SE) than those from the dispersed  
209 treatment ( $1029 \pm 28$  s SE), a difference of 2 minutes 20 seconds ( $F_{1,28} = 6.59$ ,  $P =$   
210  $0.016$ ). Copulation duration of males from the uniform treatment did not significantly

211 differ from either of the other treatments (uniform copulation duration  $1107 \pm 23$  s SE;  
212 vs. dispersed:  $F_{1,28.5} = 2.22$ ,  $P = 0.146$ ; vs. clustered  $F_{1,28.5} = 1.96$ ,  $P = 0.172$ ).  
213 However, despite these observed differences between clustered and dispersed  
214 treatments, the mean distance between males while in the treatment did not  
215 significantly affect copulation duration in any of the three treatments (all  $P > 0.101$ ).

216 In total, 159 of 180 males (88.3%) courted the female. There was no significant effect  
217 of treatment on the proportion of males that courted (generalized linear model with  
218 binomial errors and plate nested within treatment;  $\chi^2 = 118$ ,  $P = 0.376$ ). Similarly, 144  
219 (80%) of males mated, and this was not influenced by treatment ( $\chi^2 = 175$ ,  $P = 0.286$ ).  
220 Neither the latency to start courting ( $F_{2,39.3} = 0.201$ ,  $P = 0.818$ ) nor the latency to start  
221 copulation ( $F_{2,30.4} = 1.257$ ,  $P = 0.299$ ), differed significantly among the three  
222 treatments.

223

224 Discussion

225 The high degree of plasticity in mating-related traits in male *Drosophila* is well  
226 established (Churchill et al., 2019; Davies, Schou, Kristensen, & Loeschcke, 2019;  
227 Droney, 1998; Fricke, Bretman, & Chapman, 2008; Jensen, McClure, Priest, & Hunt,  
228 2015; Lefranc, 2000; Lüpold, Manier, Ala-Honkola, Belote, & Pitnick, 2010; Morimoto  
229 & Wigby, 2016; Ormerod et al., 2017; Schultzhaus, Nixon, Duran, & Carney, 2017).  
230 Variation in these traits is highly sensitive to conspecific male density in a manner  
231 which suggests that males adjust investment in anticipation of the intensity of sperm  
232 competition they are likely to encounter during mating (Bretman et al., 2009). However,  
233 how this level of plasticity relates to the variation in density and resource distributions  
234 observed in natural populations remains unknown, and laboratory studies tend to  
235 manipulate density in ways that seem unlikely to occur frequently in nature (e.g. singly-  
236 housed males compared to a high density of males in a single vial).

237 We show that manipulating food patchiness while keeping group size constant has a  
238 similar effect on a sperm competition-related trait – both in direction and magnitude –  
239 as manipulating local density directly, and that these effects can be observed even  
240 over very small spatial scales. Other studies on this species have found an  
241 approximately two-minute increase in mating duration in high density males compared  
242 to low density males (Bretman et al., 2009; Bretman et al., 2010; Bretman et al., 2013).  
243 Given that wild *D. melanogaster* encounter a patchy resource that is likely to alter male  
244 encounter rates at a similar scale to that demonstrated here (Markow, 1988; Soto-  
245 Yéber et al., 2018), we suggest that fine-scale variation in these environmental cues  
246 might influence male allocation of resources to traits associated with sperm  
247 competition, and thus mating success, in wild-living *Drosophila*.

248 Although the effect on mating duration is a repeatable indicator of male perception of  
249 sperm competition risk, the benefits of this behaviour to males remains uncertain. In  
250 many species, increased mating duration has been linked to increased sperm transfer  
251 and offspring production (Edvardsson & Canal, 2006; Engqvist & Sauer, 2003;  
252 Sakaluk & Eggert, 1996). In *Drosophila* the consequences of longer copulation  
253 durations are less clear, with some studies reporting an association with increased  
254 fitness (Bretman et al., 2009; Garbaczewska et al., 2013; Price, Lizé, Marcello, &  
255 Bretman, 2012), while others have not found a link (Bretman et al., 2012; Dobler &  
256 Reinhardt, 2016). Whether males on the clustered food resource would have a higher  
257 fitness than those on dispersed resources remains to be tested, but will almost  
258 certainly depend on mating order effects and the competing male's history of exposure  
259 to rivals (Bretman et al., 2012). However, our objective here was not to examine fitness  
260 consequences, but rather to demonstrate that males alter their perceptions of likely  
261 sperm competition risk based on small-scale changes in the spatial distribution of  
262 resources.

263 Interestingly, the effect of food distribution on male distribution behaviour and sexual  
264 investment was observed in the absence of females. Females often follow social cues,  
265 and their grouping behaviour is promoted by aggregation pheromones (Bartelt,  
266 Schaner, & Jackson, 1985; Duménil et al., 2016). By comparison, given their low  
267 feeding rate once adult (Wong, Piper, Wertheim, & Partridge, 2009), males are thought  
268 to aggregate near food resources primarily to seek mating opportunities. That these  
269 groups of males responded in their individual positioning to the distribution of food  
270 even in the absence of females is intriguing, and leaves open the question of the  
271 relative importance of female social cues compared to the direct response of males to  
272 food resources. In general however, studies manipulating male density have tended

273 to exclude females from the treatment phase (e.g. Bretman et al. (2009); Bretman et  
274 al. (2010); Lizé et al. (2012); Moatt et al. (2013); Price et al. (2012); and Rouse and  
275 Bretman (2016)), meaning the effects of inter-sexual interactions on plastic responses  
276 to density is relatively unexplored.

277 This study adds to a small number of studies that demonstrate the effects of  
278 environmental heterogeneity on *Drosophila* behaviour. Yun, Chen, Singh, Agrawal,  
279 and Rundle (2017) demonstrated that female fitness was higher in more spatially  
280 complex laboratory environments as a result of a reduction in sexual interactions and  
281 consequent mitigation of male harm. Similar effects have been demonstrated when  
282 laboratory populations were presented with a refuge: female remating rates declined  
283 substantially (Byrne, Rice, & Rice, 2008). Such rapid shifts in behaviour, driven by  
284 ecological patchiness, have rarely been included in laboratory assays, but may have  
285 major effects on the demography and growth rate of populations exposed to spatial  
286 patchiness, through their effects on male reproductive skew and therefore effective  
287 population size. Such effects may have important evolutionary and ecological  
288 consequences in relatively patchy parts of a species' distribution, for example by  
289 increasing sexual conflict over shared resources (Pilakouta, Richardson, & Smiseth,  
290 2016), or reducing maximum sustainable rates of evolution (Bridle, Kawata, & Butlin,  
291 2019; Bridle, Polechová, & Vines, 2009).

292 There are some intriguing dynamics operating in the inter-male distances in the early  
293 stages of the treatment period: in particular, males on the dispersed food patches  
294 initially experience lower inter-male distances than those on the clustered food (Fig.  
295 1). This effect does not match what we expected to see among males attempting to  
296 defend individual patches, and is the opposite to the pattern observed on the final days

297 of treatment. Inspection of photographs from this treatment suggests that males on  
298 the dispersed food patches initially cluster together away from food before sorting  
299 themselves into individual territories focussed around each patch. Territorial behaviour  
300 in *D. melanogaster* has previously been observed under laboratory conditions, and  
301 appears to be driven by boundaries of food sources (Lim, Eyjólfsdóttir, Shin, Perona,  
302 & Anderson, 2014) so it is possible that multiple distinct territories could be established  
303 under these conditions. However, it remains unclear what is driving the initial clustering  
304 behaviour.

305 Our results demonstrate a clear link between small-scale patchiness of resources and  
306 sexual behaviours that suggest that males are sensitive to sperm competition risk,  
307 mediated by changes in male-male encounter rate. While density effects on male  
308 mating duration have been demonstrated several times, we have placed this response  
309 in a biologically meaningful context by demonstrating a link to ecological factors that  
310 are very likely to be at play in wild-living populations.

311

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317

318 Author contributions

319 ERC, MDFT, and JRB designed the study; ERC performed all experiments; ERC and

320 MDFT analysed the data; all authors contributed to writing the manuscript.

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490

491 Figure 1. Mean inter-fly distance (mean of 6 pairwise distances between 4 focal flies  
492 per plate, averaged across 11 replicate plates) over time. Black = uniform treatment  
493 (evenly distributed food); red = clustered food patches; blue = dispersed food patches.  
494 Bars show standard errors of the mean for each time point across all 11 treatment  
495 replicates. Grey blocks indicate period of dark (2000 - 0800 GMT), and are not to  
496 scale.

497

498 Table 1. Details of statistical parameters from linear mixed models analyses outlined  
499 in the results. Model outputs are presented in the order they appear in the text.

500 Response variables and data subsetting are outlined in the subheadings, predictor  
501 variables in the 'Parameter' column.

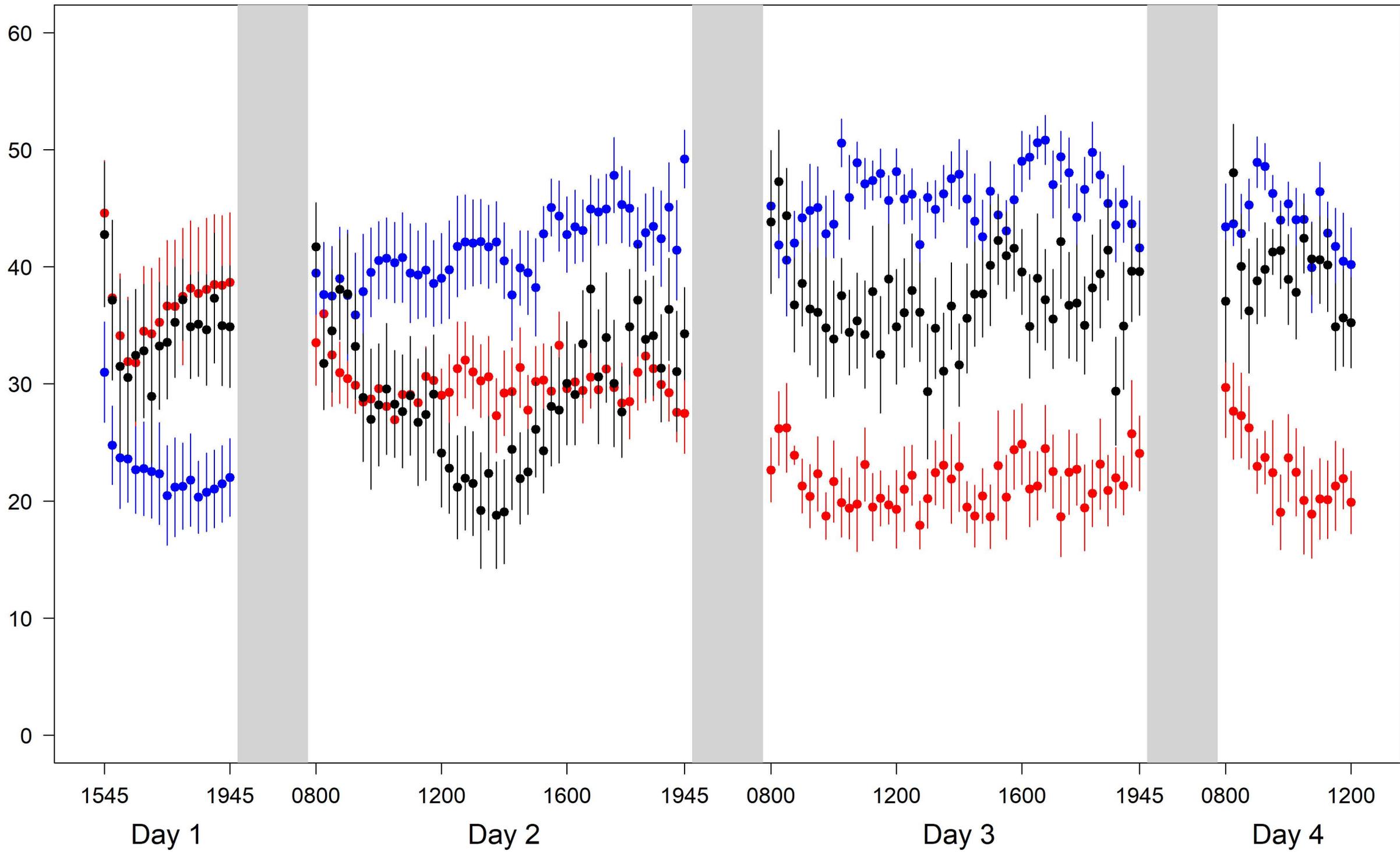
502

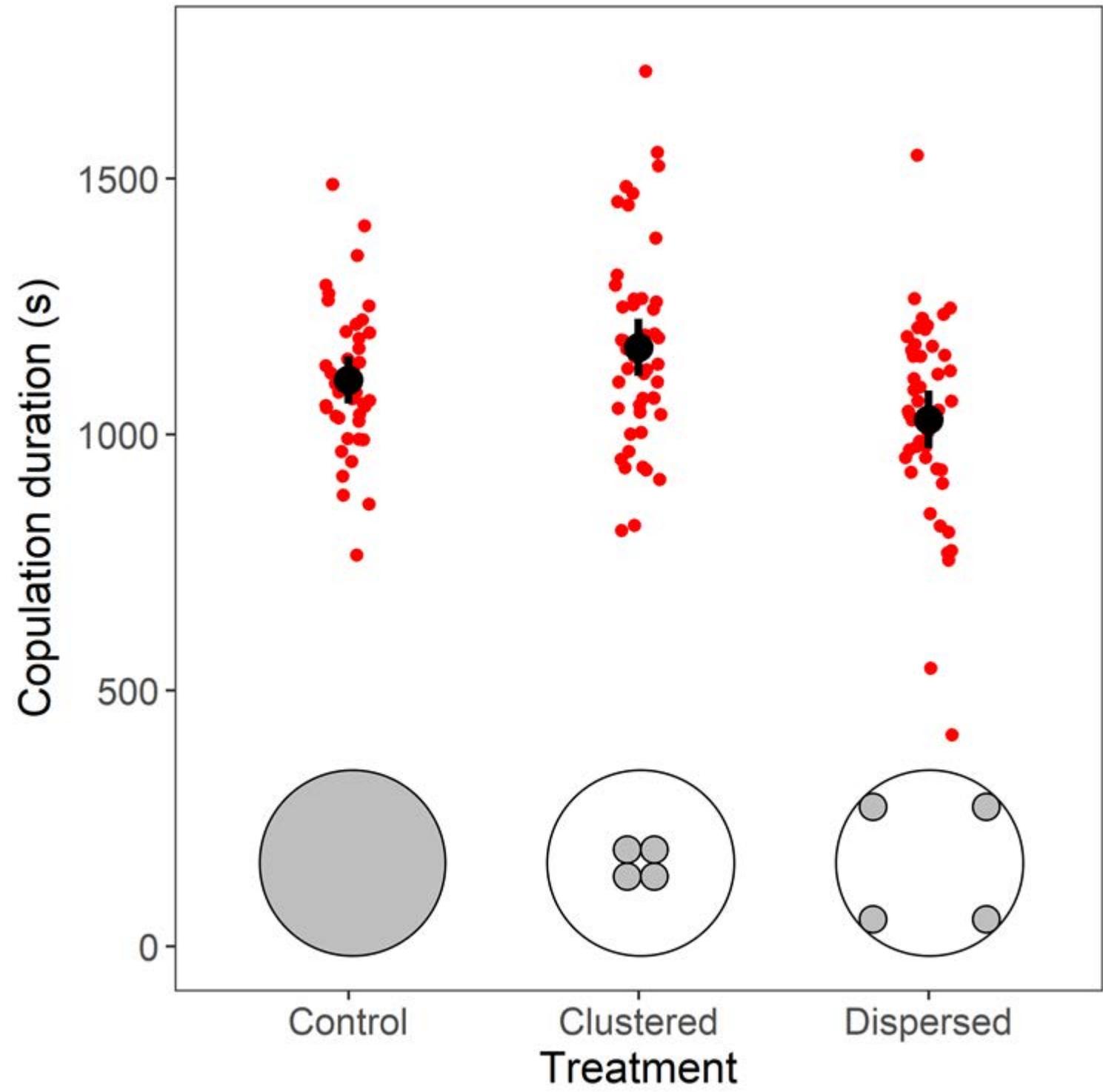
503 Figure 2. The effect of food resource spatial distribution on the duration of subsequent  
504 copulation. Means (black dot) and 95% confidence intervals of copulation duration  
505 (seconds). Sample sizes: clustered 49 (11 males did not mate), uniform 44 (16),  
506 dispersed 51 (9). The treatment effect on mating duration remains significant when the  
507 two mating duration values below 600s in the dispersed treatment are excluded from  
508 the analysis ( $F_{2,40.9} = 3.55$ ,  $P = 0.038$ ).

509

510

Mean distance between individuals (mm)





<b>Parameter</b>	<b>Estimate</b>	<b>SE</b>	<b>T</b>	<b>p</b>
<b><i>Pairwise distance between males: full duration of treatment</i></b>				
Clustered (intercept)	35.14	1.85	18.978	<0.0001
Uniform	-6.305	2.618	-2.408	0.021
Dispersed	-3.930	2.617	-1.501	0.142
Time sequence	-0.127	0.008	-14.946	<0.0001
Uniform*time	0.207	0.012	17.225	<0.0001
Dispersed*time	0.276	0.012	23.025	<0.0001
<b><i>Pairwise distance between males: final day of treatment<sup>a</sup></i></b>				
Clustered (intercept)	22.794	1.983	11.493	<0.0001
Uniform	16.560	2.777	5.963	<0.0001
Dispersed	21.224	2.777	7.643	<0.0001
<b><i>Copulation duration</i></b>				
Clustered (intercept)	1170.9	35.28	33.19	<0.0001
Uniform	-64.7	51.12	-1.266	0.2124
Dispersed	-140.31	49.89	-2.813	0.0075
<b><i>Copulation duration; outliers removed<sup>b</sup></i></b>				
Clustered (intercept)	1170.55	31.98	36.60	<0.0001
Uniform	-64.45	46.46	-1.387	0.173
Dispersed	-121.13	45.48	-2.66	0.0112
<b><i>Courtship latency</i></b>				
Clustered (intercept)	925.5	176.37	5.247	<0.0001
Uniform	-157.78	249.9	-0.631	0.531
Dispersed	92.17	245.2	-0.376	0.709
<b><i>Copulation latency</i></b>				
Clustered (intercept)	954.33	183.00	5.215	<0.0001
Uniform	-254.07	262.09	-0.969	0.340
Dispersed	154.10	255.73	0.603	0.552

a non-significant time\*treatment term removed

b two outliers in the dispersed treatment with copulation duration values < 600 seconds removed