

1 Honest cues contribute to male choice for 2 female guarding in a herbivorous spider 3 mite 4

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16 Abstract

17 Mate choice is a wide-spread phenomenon with important effects on ecological and evolutionary
18 dynamics of successive generations. Increasing evidence shows that males can choose females if
19 females vary in quality and these mating choices can strongly impact fitness. In the herbivorous spider
20 mite *Tetranychus urticae* males engage in precopulatory mate guarding of quiescent females, and it is
21 known that females vary in their time to sexual maturity and fecundity. However, our understanding
22 of how males maximize their reproductive success and which female phenotypic traits are important
23 cues for their mating decisions are still limited. In many arthropod species, female body size and
24 pheromones are well known proxies for fecundity. These traits—and thus possibly male mating
25 decisions—are however sensitive to environmental (dietary) stress. By allowing males to freely choose
26 amongst many (synchronized) females in a controlled semi natural environment, we found that
27 guarded females have a higher fecundity and are closer to sexual maturity than non-guarded females.
28 Despite the fact that female body size was positively correlated with fecundity and significantly
29 influenced by the environment, males did not discriminate on body size nor did we find evidence that
30 they used other cues like cuticular pheromones or copying behavior (social cues). In conclusion we
31 were able to show male mate preference for females that are closer to sexual maturity and have higher
32 fecundity, but we were unable to identify the female traits that signal this information

33

34 Introduction

35 In animals, mate choice can be defined as the specific behavior by which the quality of potential mates
36 is assessed before copulation or fertilization (Bateson, 1983; Edward, 2014). The paradigm that
37 females are a limiting factor in animal reproduction because of costly eggs (Bateman, 1948) is an
38 erroneous simplification of complex eco-evolutionary processes (Edward & Chapman, 2011; Tang-
39 Martínez, 2016). Males of many animal species provide nuptial gifts, invest in lengthy courtship or
40 mate guarding, further reducing the number of future copulations (Kvarnemo & Simmons, 1998;
41 Vahed, 1998; Byrne & Rice, 2006; Han & Jablonski, 2010; Papadopoulos et al., 2010; Oku, 2014). Such
42 investment costs for males, favor male choosiness if female quality varies and this quality can be
43 perceived by the males (Snowdon, 1997; Bonduriansky, 2001; Edward & Chapman, 2011; Hare &
44 Simmons, 2019).

45 In many arthropods it is known that males prefer females with larger body size as it is associated with
46 larger reproductive output (Honěk, 1993; Calvo & Molina, 2005; Drapela et al., 2013). Males use visual
47 and/or tactile cues to evaluate female body size and adjust their mating behavior according to the
48 estimated size (Gage, 1998; Byrne & Rice, 2006; Chenoweth et al., 2007). Chemical signals such as
49 pheromones can signal mating status and sexual maturity and equally provide honest information
50 about female fecundity (Gaskett, 2007; Steiger & Stökl, 2014). These chemical cues may be
51 differentially perceived depending on environmental conditions or distances from the potential mate
52 (Bel-Venner et al., 2008). Besides gathering information about female quality directly, individuals may
53 use social cues about potential mates as well. In this way, males can save time and energy by copying
54 other individuals instead of thoroughly investigating the female quality themselves (Scauzillo & Ferkin,
55 2019). Such a choice copying is considered to be context-dependent (Witte, Kniel, & Kureck, 2015),
56 leading to contrasting results in series of empirical studies with respect to the strength and reliability
57 of female signals (Gaskett, 2007). This is because strong manipulative studies have their own
58 constraints and introduce biasing effects, rendering insights on the relevance and significance of mate
59 choice elusive. Many choice test, for instance, overestimated mate preference (Dougherty & Shuker,
60 2015).

61 We here aimed to avoid such constraints by testing mate selection in the two-spotted spider mite
62 *Tetranychus urticae* under semi-natural conditions. *T. urticae* guard females that are about to molt to
63 the adult stage (the quiescent female deutonymphs, or teliochrysalids). This guarding behavior allows
64 males to secure the first mating opportunity and fertilize all the eggs of the guarded female (commonly
65 referred as first sperm precedence; (Helle, 1967). Guarding is time-consuming and exposes spider mite
66 males to predators, diseases and competitors (Alcock, 1994; Lima, 1998; Oku & Yano, 2008; Oku, 2009)
67 and induces aggressive interactions among males (Potter, Wrensch, & Johnston, 1976), potentially
68 result in wounding or even death (personal observations SG, NW; Potter, Wrensch, & Johnston, 1976).
69 Therefore, males employing precopulatory mate guarding and fighting are anticipated to maximize
70 their reproductive success by choosing the most fit females to guard. Males tend to select
71 teliochrysalids that are close to emerge hereby minimizing the time invested per copulation (Parker,
72 1974; Everson & Addicott, 1982). Despite this intuitive appeal of the time minimization hypothesis,
73 later studies generated inconsistent results in *T. urticae* (Saito, 2010). These non-consistent results
74 may result from varying environmental conditions especially the population density and sex-ratio, as
75 this directly affects the level of mate competition (Macke et al., 2012).

76 Consistent with studies that uncovered a limited visual capacity of *T. urticae* (Naegele, McEnroe, &
77 Soans, 1966; W. D. McEnroe & Dronka, 1966; William D. McEnroe, 1969), chemical sensing of foliar
78 structures and webbing have been implicated as important determinants in male searching behavior
79 for female teliochrysalids (Cone et al., 1971; Penman & Cone, 1972). Some evidence on the importance

80 of sex-pheromones has been provided in the 70ies of the previous century (Regev & Cone, 1975, 1976,
81 1980), but their effective function in signaling remains elusive and no volatile pheromones have been
82 discovered that are known to contain information about the condition or quality of potential partners
83 (Royalty, Phelan, & Hall, 1993; Oku, 2014; Oku et al., 2015). There is some evidence of mate choice
84 copying behavior in spider mites, with males preferring guarded over solitary females, most likely
85 because the former invest into a higher pheromone production (Oku & Shimoda, 2013). Paradoxically,
86 solitary females had the highest fecundity, most likely because of being released from this costly
87 pheromone production. This mating strategy therefore increases female fitness by attracting the most
88 fit males, but not male fitness (Biernaskie, Grafen, & Perry, 2014). Given the fast resource exploitation
89 dynamics of the species, dispersal rates (and therefore the potential to monopolize new resources) are
90 an important fitness-related measure as well (Massol, Calcagno, & Massol, 2009; Bonte & Dahirel,
91 2017).

92 In this study, we observed mate guarding behavior of *T. urticae* under low and high population density
93 in semi-natural conditions where the males can assess many different females before their mate
94 choice. This allowed us to separate guarded and unguarded females under the different environmental
95 conditions, and enables us to document putative alternative mating tactics of males. There is some
96 evidence that body size is positively correlated with fecundity in this species (Li & Zhang, 2018). It has
97 been shown that the CHC profiles of several insect species serve as indicators of female fertility as well
98 (Thomas & Simmons, 2010; Bilen et al., 2013; Smith & Liebig, 2017), and that they may even be
99 genetically correlated with fecundity (Berson & Simmons, 2019). In contrast to volatile pheromones,
100 such CHC's have not been investigated in spider mites. Lastly, we tested if males copy conspecific males
101 by evaluating the spatial configuration of guarded females on the total leaf. We suspect that copying
102 behavior and possible clustering of teliochrysalids could result in aggregations of guarded and
103 unguarded females. We evaluated potential fitness maximization, and therefore the honesty of cues,
104 by quantifying life history traits of all guarded and non-guarded females.

105

106 Materials and Methods

107 Mite husbandry

108 The *T. urticae* strain Lede was used in this study and is derived from the reference laboratory strain LS-
109 VL that was originally collected from roses in Ghent, Belgium (Van Leeuwen, Stillatus, & Tirry, 2004).
110 Mites of the stock population were reared on potted bean plants (*Phaseolus vulgaris* L. cv. 'Prelude')
111 in a climate-controlled room at 26 ± 0.5 C, 60 % RH and 16/8 h (L/D) photoperiod. To create high (HD)
112 and low (LD) densities, 40 (HD) and 10 (LD) adult females were randomly collected from the stock
113 populations and transferred to leaf discs of 3.5×4.5 cm², (freshly cut from 2-week-old plants) on wet
114 cotton in an incubator (27 °C, 16:8 LD). Females were subsequently allowed to oviposit for 24 hours.
115 On the 40 HD leaf discs there were an average of 15 eggs/cm², whereas 40 LD leaf discs held on average
116 2 eggs/cm². After 7 days left to develop in an incubator (37°C, 16:8 LD) most of the female mites were
117 in the teleochrysalids stage (nymphal females in their final quiescent stage). On these leaf discs with
118 females in the teleochrysalids stage, most male spider mites already reached adult hood and are
119 present in a ratio of 1:3 (one male for every 3 females). Some males guard quiescent deutonymph
120 females, the stage immediately before adult emergence and sexual maturation. The duration of the
121 quiescent deutonymph stage is ca. 1 day at 27°C with a L16:D8 photoperiod (Kabiri, Saboori, &
122 Allahyari, 2012). Immediately after emergence the guarding male quickly copulates with the adult
123 female. The experiments were conducted in 2 batches, with a total of 35 HD,LD plates in the first set
124 ad 35 HD, LD plates in the second experiment.

125 We let the males choose freely among many different teleochrysalids and subsequently measured
126 their spatial clustering. Fitness related traits were measured by subsequently transferring individual
127 guarded and unguarded teleochrysalids to bean leaf discs (2×2 cm²) that were connected by parafilm
128 bridges on top of moist cotton and were lined with 2-mm-wide strips of moist tissue paper. For both
129 the HD and LD treatments, 30 guarded and unguarded teliochrysalids were selected from random
130 plates in both experiments, making a total of 240 individuals measured for body size, fecundity,
131 survival and dispersal. After measuring the teliochrysalids body size the guarding male was introduced
132 on the leaf disc to fertilize the emerging female, a random male was introduced on leaves of non-
133 guarded teleochrysalids. In the second experiment no males were introduced on the leaf disc and thus
134 all females are not mated. Time till emergence was tested for mites from the two batches of
135 experiments, with respectively 38, 29 and 26 guarded females and 35,27 and 28 unguarded females
136 for both experiments. Teleochrysalid cuticular chemical profiles were quantified from pools of 30
137 guarded and unguarded teleochrysalids from experiment 1.

138

139 Spatial clustering of guarded females

140 We allowed all males from the age-synchronized populations to choose female teleochrysalids on the
141 leaf discs and subsequently analyzed the spatial configuration of guarding males. If males were
142 attracted by other males (through social cues), we expected a clustering of guarded teleochrysalids on
143 the bean leaves.

144 To obtain the spatial configuration of guarding males, the seven day old leaves were photographed in
145 a standard way of six leaves (3 HD and 3 LD). The positions of the guarded and unguarded
146 teleochrysalids were marked using ImageJ (Schneider, Rasband, & Eliceiri, 2012) and were confirmed
147 by manual inspection under a stereo microscope. After one hour, leaf discs were screened again, which
148 confirmed that all females marked as guarded had remained guarded. The spatial positions were
149 analyzed as point patterns with Spatstat (Baddeley, Rubak, & Turner, 2015). To analyze if guarded
150 females were more clustered in the point pattern, the mark equality function is calculated for the six
151 patterns. This function gives the probability that two points have the same mark (guarded or
152 unguarded) when separated by a distance r . If guarded females are more clustered, this function would
153 be higher at small distances than complete spatial randomness (horizontal line at value 1). Acceptance

154 regions were drawn around the complete spatial random line using Monte Carlo simulation envelopes
155 (n=500) to infer if the mark equality functions differ from complete spatial randomness.

156

157 **Fitness related traits in guarded and unguarded mites**

158 *Body size*: we measured the teleochrysalid abdomen surface area (Troscianko, 2014) from high-
159 resolution photos (Leica M50, 5 MP HD Microscope Camera Leica MC170 HD).

160 *Fecundity*: we tested whether male guarding was related to female reproductive performance by
161 quantifying the number of offspring from a single females after 12 days (Wybouw et al., 2015).

162 Body size and fecundity were analysed by means of a linear mixed effect (lme) model in R using the
163 package 'lme4'. Mother density treatment, guarding status and their interaction were used a
164 explanatory variables. We also included the iteration of the experiment as a variable intercept. Post-
165 hoc analysis was performed on the estimated marginal means of the lme using the 'emmeans' package.

166 *Survival*: female mortality was monitored during a period of 20 days. We fitted a mixed-effect Cox
167 model of how female mortality in time depended on whether the female was guarded or not. Females
168 that died in the first 24 minutes were omitted because early deaths are attributed to the experimental
169 manipulation. The mixed-effect Cox model enabled us to control for the possible auxiliary effects that
170 were present during the two repetitions by including it as a variable intercept. We verified the
171 proportional hazard assumption on each experiment separately (Supplementary statistics ,2).

172 *Time till emergence*: We tested whether males could perceive time to emergence when deciding which
173 female to guard. Teleochrysalids that were guarded or unguarded for at least one hour (see marking
174 for the spatial analysis) were individually transferred to bean leaf square (4 x 4 cm²). The leaf was
175 divided in two half's and guarded females were placed on one side and the unguarded on the other. A
176 series of time laps pictures was taken (Leica M50, 5 MP HD Microscope Camera Leica MC170 HD) every
177 10s for 24h. The pictures of the time laps were analyzed to determine the molting time of each guarded
178 and unguarded female.

179 We fitted a mixed-effect Cox model of how female emergence in time depended on whether the
180 female was guarded or not using the 'survival' and 'coxme' packages (Therneau et al., 2021). The
181 mixed-effect Cox model enabled us to control for the possible auxiliary effects that were present
182 during the three repetitions by including it as a variable intercept. Data from the first experiment
183 violated the proportional hazard assumption and were excluded from the formal analyses (but
184 provided in supplement statistics,1a,1b).

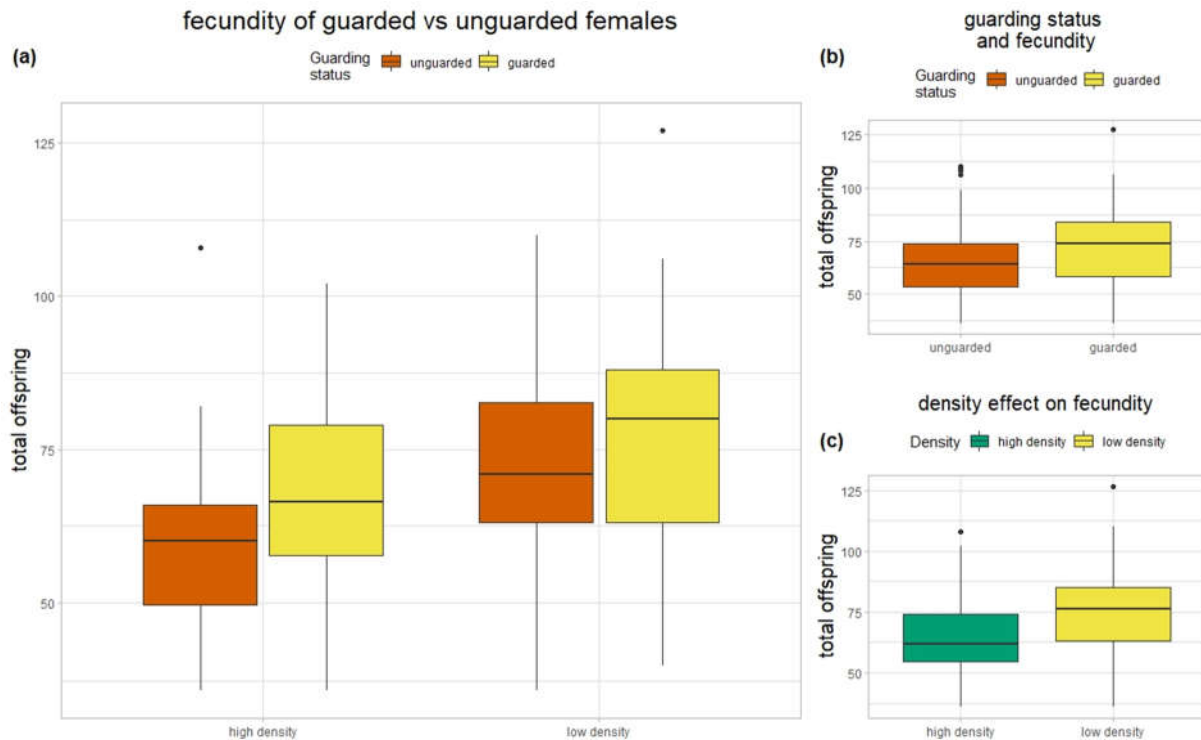
185 *Dispersal*: We quantified dispersal by the movement of mites across parafilm bridges to adjacent leaf
186 discs (Dahirel et al., 2019) for adult females in the first batch of experiments. Spider mite movements
187 were monitored 4 times/day. We constructed a Kaplan-Meier model of how dispersal in time
188 depended on whether females were previously guarded or not and on their density during testing. The
189 survival and dispersal analyses were conducted with the 'survival' and 'coxme' packages (Therneau et
190 al., 2021).

191

192 **Chemical profile of guarded and non-guarded females**

193 Here, we wanted to test whether the teleochrysalid cuticular chemical profile affected male guarding
194 behavior. We transferred pools of 30 guarded and unguarded teleochrysalids with hexane-cleaned
195 brushes to separate 2-mL glass vials in five replicates (Sigma-Aldrich). In addition, we also transferred
196 pools of 30 adult males to separate 2-mL vials in two replicates. The teleochrysalids and adult males
197 were frozen and kept at -21 °C until solvent extraction and GCMS analysis. We extracted the
198 cuticular compounds for 10 min in 2-mL vials capped with a PTFE septum (Sigma-Aldrich) in 20 µL of

199 hexane (HPLC grade, Sigma-Aldrich). The hexane extract was transferred to another vial. Samples
200 were left to evaporate at room temperature in a laminar fume hood and stored at -21°C prior to
201 analysis. Samples were diluted again in $20\text{-}\mu\text{L}$ hexane. We injected $2\ \mu\text{L}$ of each hexane extract into a
202 Thermo GC (Trace 1300 series) coupled with a MS (ISQ series, $-70\ \text{eV}$, electron impact ionization) and
203 equipped with a Restek RXi-5sil MS column ($20\ \text{m} \times 0.18\ \text{mm} \times 0.18\ \mu\text{m}$). Extraction, identification
204 and quantification of the CHCs was conducted as described in (Parmentier et al., 2018).



205

206 **Figure 1:** Fecundity of guarded females and unguarded females in high and low density plots. (a) fecundity (total offspring)
207 of the guarded females is significantly higher in high (food stress) and low density conditions. (b) Total difference in
208 fecundity of unguarded vs guarded females. (c) Total difference in fecundity between high density (food stress) and
209 low density conditions.

210 Results

211 Spatial clustering of guarded females

212 Mark equality plots of the three HD and three LD point patterns showed that guarded (or unguarded)
213 females are not more clustered together than expected under complete spatial randomness
214 (Supplementary fig. S1).

215 Fitness related traits in guarded and unguarded mites

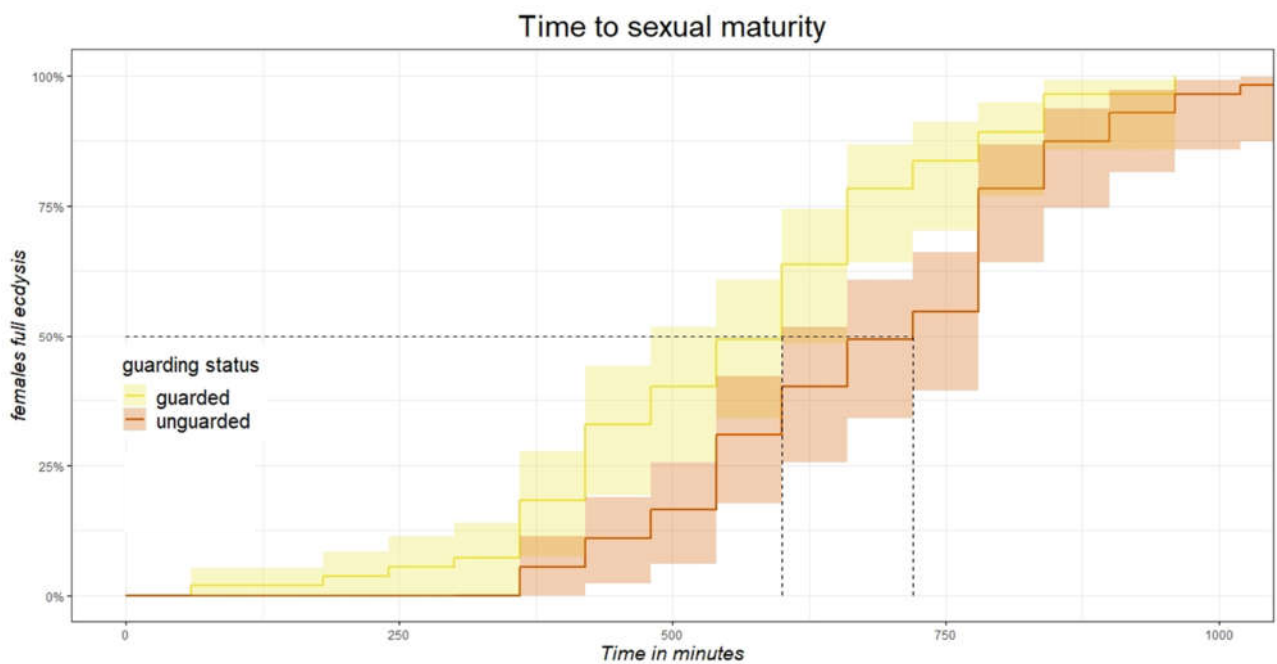
216 Female body size did not differ significantly between the guarding status ($247 \pm 14.6\ \mu\text{m}$ for unguarded
217 individuals, $243 \pm 14.6\ \mu\text{m}$ for guarded individuals, difference: $3.51\ \mu\text{m}$, $p = 0.1688$, supplementary fig.
218 S2). As expected from the more stressful high density treatment, female mites exposed to a high
219 density were significantly smaller ($t(\text{density}) = 4.692$, $p < 0.0001$) than those mites kept in low density
220 ($237 \pm 14.5\ \mu\text{m}$ for HD individuals versus $253 \pm 14.6\ \mu\text{m}$ for LD individuals, difference: $-16.1\ \mu\text{m}$, $p < 0.0001$,
221 Supplementary fig. S2). The large difference in body size between HD and LD mites and the variation
222 within the treatments shows that a variation in body size can be found, but in our experiment males
223 did not prefer larger females.

224 Body size was positively correlated to fecundity ($r = 0.30$; $p = 0.00018$, Supplementary fig. S3). The total
225 number of offspring (fig. 1, panel a) differed between density treatments and between the guarding

226 status of the female. Females were estimated to produce 63.2 ± 3.78 offspring on high density leaves
227 versus 73.1 ± 3.82 offspring on low density leaves (difference: -9.98 , $p < 0.0001$, fig. 1, panel c).
228 Furthermore, guarded females produced 71.6 ± 3.78 offspring, unguarded females 64.7 ± 3.79 offspring
229 after 12 days (difference: 6.83 , $p = 0.0014$, Fig. 1, panel b). We did not observe a significant interaction
230 effect between mother density and guarding.

231 Over both analyzed experiments, guarded teliochrysalids molted to adult females with a probability
232 that was 1.82 higher than that of unguarded females (Cox mixed-effects model, $\exp(\text{coeff}) = 1.82$,
233 $p = 0.0023$; Fig 2). Before 900 min (15h) we see at each time point that a higher proportion of guarded
234 females emerged but after 900 min this levels out. Males seem therefore able to detect females that
235 will emerge from their final ecdysis within 900 min.

236 The Kaplan–Meier estimator shows that there were no differences in survival ($p = 0.48$), or dispersal
237 ($p = 0.75$) between guarded and non-guarded females (see supplement statistics, 2a, 2b, 3).



238

239 **Figure 2** : Time to event (sexual maturity) plot of guarded and unguarded females. Guarded females emerge faster from
240 there resting state than unguarded females with the largest differences at the early time points (faster emergence) and less
241 difference past 800 minutes (33h).

242

243 Chemical profile of guarded and non-guarded females

244 The cuticular hydrocarbons of the spider mites constituted a homologous series of saturated straight
245 chain alkanes from n-C22 to n-C33 (supplementary Figures, S4). There were quantitative differences in
246 the CHC composition between males and females, males formed a separate group in an nMDS plot
247 (Permanova—adonis, 999 permutations, Pseudo-F = 7.77, $R^2 = 0.6334$, $P = 0.008$). but guarded females
248 could not be separated graphically in an nMDS plot (supplementary Figures, S4). Consequently, no
249 effect of treatment group on female CHC composition could be demonstrated (Permanova—ADONIS,
250 999 permutations, Pseudo-F = 1.04, $R^2 = 0.112$, $P = 0.439$).

251

252 Discussion

253 In this study, integrated experiments show that *T. urticae* males preferred to guard females that
254 differed in sexual maturation and fecundity from unguarded females. Contrary to our hypotheses we
255 did not find any evidence that males use cuticular hydrocarbons, body size or social cues in sexual
256 selection.

257 As in other species (e.g., amphipods; Dick & Elwood, 1990), spider mite males likely rely on cues that
258 provide information on both fecundity and time till maturity to enhance their mating success.
259 Alternatively, mate guarding could shorten the female quiescence, so advance maturation in females
260 rendering the presence of males rather the cause than the consequence of life history changes. Indeed,
261 as for amphipods (Ward, 1984), spider mites females have been shown to delay molt in absence of
262 mating partners (Oku, 2016).

263 We found that guarded females were more likely to be closer to molting than unguarded females with
264 indications that small differences in molting in the last 15h to molting are detected. Conversely, Oku
265 & Saito (2014) also found that males preferred females close to molting but could only discriminate if
266 the time to molting was more than 22h apart. This can be explained by the fact that males in our
267 experiment could assess all possible options, and reject females closer to maturation in favor of
268 females that have the energy and resources to lay more eggs but take a bit longer to molt. Also some
269 females close to maturation could already be guarded by other males, so males have to waste time
270 and energy for male combat to take over the most optimal females or search for the next best female
271 that is not guarded. By using both the time they have to invest in guarding and female fecundity, males
272 can maximize their reproductive gains per unit time of investment. It is known that males usually do
273 not spend much time in inter-male conflicts (<5s), with the approaching male being attacked and
274 driven off by a guarding male in most cases (Royalty, Phelan, & Hall, 1993). In our experiment we also
275 found that once males become arrested near a female, they tend to commit to guard that female until
276 they mate with the guarded female. Alternatively, males can also vary in their preference and ability
277 with no single female “type” favored by all males. It would therefore be interesting to study male traits
278 in association with their choice and competition for specific female (traits). Moreover, we argue that
279 male mate choice may be a threshold response where male investment is conditional to the available
280 energy budget, density of females, other males, the time until the female reaches sexual maturity and
281 total number of offspring per female.

282 The positive correlation between body size of the teleochrysalids and fecundity was rather modest
283 (0.3) and males in our experiment did not prefer or could not distinguish the larger females. We did
284 find that in the lower stress condition (low density), females were significantly larger than females
285 raised in a stressful environment (high density). However, male choice for body size was not influenced
286 by the environment. Similarly, females in the low stress conditions had significantly more offspring
287 than the females in the stressful environment. Although males generally prefer larger females in many
288 insects and spiders (e.g., Saeki, Kruse, & Switzer, 2005), females may also “chose” to allocate resources
289 to reproduction instead of other traits such as growth and have therefore body size decoupled from
290 fecundity. In these, large body size is not selected for (e.g., Wolz et al., 2020) or even counter-selected
291 (e.g., Klingenberg & Spence, 1997).

292 A positive relation between fecundity and cuticular hydrocarbons (CHC's) is found in many insects and
293 quantitative and qualitative differences in CHC's are important in for partner choice in spiders (Gaskett,
294 2007; Chung & Carroll, 2015; Berson & Simmons, 2019). In spider mites, volatile pheromones have long
295 been investigated as an attractant for male spider mites, with sometimes contradicting results (Oku,
296 2014). Even though it is possible that males are attracted by volatile pheromones, it seems unlikely
297 that these volatiles found in spider mites so far provide information about the quality of the female
298 (Saito, 2010; Oku et al., 2015). As in spiders we suspected that airborne sex pheromones typically
299 attract males, but rarely elicit courtship (Gaskett, 2007). In contrast CHC's not only provide males with
300 information about female fecundity but in many species their composition also changes with sexual

301 maturation (Ala-Honkola et al., 2020). Although previous studies used proxies to investigate the
302 attraction of male spider mites to CHC's and distinguish these from the attraction to volatiles
303 (Rodrigues et al., 2017), no studies on their role as a possible cue for female quality exist. We found
304 that cuticular CHC's composition of *T. urticae* consists of a homologous series of saturated straight
305 chain alkanes from c22 to c33. A similar result of CHC's arranged in a series of straight chain alkanes
306 was found in oribatid mites (Acari: Oribatida) (Rasputnig et al., 2008). We could not find any differences
307 in quantity nor quality of the CHC's between guarded and unguarded females. As a consequence of
308 small sizes of the spider mites, and thus, minute amounts of hydrocarbons, a high number of
309 individuals was necessary to prepare crude extracts with detectable alkane-profiles. This could also
310 mean that a more subtle signal of female quality might not be noticed by our methods as we cannot
311 evaluate every female separately. Alternatively, pheromones signaling female quality and sexual
312 maturation could also be present in the webbing of the resting females as is known from spiders
313 (Baruffaldi et al., 2010; C. Scott et al., 2018). Although it is suggested that spider mites can use silk as
314 a movement cue, nothing is known about what information is present in the silk (Penman & Cone,
315 1974). For instance during web production female spider can deposit large amounts (up to 5µg/web)
316 of pheromones in their silk, providing possible mates with cues about their condition (Schulz & Toft,
317 1993; Gaskett, 2007).

318 Finally, we did not find any evidence that males use social cues in guarding behavior. Although there
319 were multiple teliochrysalids close to each other, mostly only one or two of these were guarded. The
320 use of social information in mating is not uncommon in spiders and insects (Mery et al., 2009; Fowler-
321 Finn et al., 2015; C. E. Scott, McCann, & Andrade, 2019). Relying on social information, mate-choice
322 copying, and thus aggregating close to deceptive females, is costly as it increases conflict and direct
323 mortality (Potter, Wrensch, & Johnston, 1976). Hence, male mate-choice copying behavior is only
324 expected to occur when searching (time) costs are high (Real 1990). In spider mites, searching times
325 are likely not very high as mating partners are quite densely distributed. This contrasts strongly with
326 systems where such behavior was observed, e.g. in spiders where mate searching can have high
327 mortality rates, favoring efficient detection of and movement towards females (C. E. Scott, McCann, &
328 Andrade, 2019). Our results indicate that males avoid to guard females that are attended by a number
329 of males. We cannot rule out that there was conflict between males before they settled in their final
330 choice, but in all cases there were still more females unguarded than guarded, leaving ample variation.
331 We neither found any adjustment of these mate selection strategies over the two density treatments,
332 despite changes in phenotypes.

333 Although previous research found that spiders mites changed their guarding behavior in response to
334 density we did not find any indication that males changed their guarding behavior (Oku, 2009). We did
335 find that the body size and fecundity differed for the females between our density treatments
336 indicating an effect of the stressful conditions. Nevertheless it is possible that our 'high density' was
337 not high enough for the males to alter their guarding behavior.

338 In conclusion, we here demonstrated non-random mate selection of spider mites in semi-natural
339 settings. Our work complements earlier work using choice experiments and shows that even in
340 conditions where multiple cues are present, that sexual selection is based on honest cues, i.e., that
341 males select females directly providing largest fitness benefits by selecting females that minimize time
342 costs (guarding time) as well as maximizing reproduction. This signaling appears to be based on other
343 cues than cuticular hydrocarbons, body size or social information.

344

345

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353 Author contributions: The study was initially conceptualized by S.G. and developed with input from
354 D.B. and T.P. .S.G developed the experiments and collected data. T.P. extracted the cuticular
355 hydrocarbons (CHC's) and conducted gas chromatography and subsequent identification and statistical
356 analysis of the CHC's. S.G and F.M. performed the statistical analysis of life history traits and body size.
357 FB performed the spatial analysis. S.G. and N.W. drafted the manuscript. All authors proofread the
358 manuscript and made valuable contributions.

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