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## Rapid turnover of a pea aphid superclone mediated by thermal endurance in central Chile

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1  
2 Running title: Extensive pea aphid superclone turnover

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5 **Rapid turnover of a pea aphid superclone mediated by thermal**  
6 **endurance in central Chile**

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25 **ABSTRACT**

26 Global change drivers are imposing novel conditions on Earth's ecosystems at an  
27 unprecedented rate. Among them, biological invasions and climate change are of critical  
28 concern. It is generally thought that strictly asexual populations will be more susceptible to  
29 rapid environmental alterations due to their lack of genetic variability and, thus, of adaptive  
30 responses. In this study, we evaluated the persistence of a widely distributed asexual  
31 lineage of the alfalfa race of the pea aphid, *Acyrtosiphon pisum*, along a latitudinal transect  
32 of approximately 600 Km in central Chile after facing environmental change for a decade.  
33 Based on microsatellite markers, we found an almost total replacement of the original aphid  
34 superclone by a new variant. Considering the unprecedented warming that this region has  
35 experienced in recent years, we experimentally evaluated the reproductive performance of  
36 these two *A. pisum* lineages at different thermal regimes. The new variant exhibits higher  
37 rates of population increase at warmer temperatures, and computer simulations employing  
38 a representative temperature dataset suggest that it might competitively displace the original  
39 superclone. These results support the idea of a superclone turnover mediated by differential  
40 reproductive performance under changing temperatures.

41

42 **Keywords:** aphid superclone, heat tolerance, intrinsic rates of population increase,  
43 microsatellite

## 44 1. INTRODUCTION

45 As global environmental conditions change rapidly, they threaten biodiversity significantly,  
46 leading to an uncertain future for living systems [1, 2]. While adaptive responses to rapid  
47 changes might be anticipated, identifying which species and populations would be capable  
48 of adapting and the speed at which they could do so remain challenging in the current  
49 context of global change [1 - 5]. Among global change drivers, biological invasions and  
50 climate change represent two of the most critical threats to biodiversity [1, 2, 6]. Indeed,  
51 biological invasions are now considered one of the “pillars” of biological extinctions [7], being  
52 facilitated to some extent by climate change via the rise in environmental temperatures and  
53 increased frequency of extreme climatic events [2].

54 Under the current scenario of accelerated environmental changes, we could  
55 anticipate problematic effects of global change on asexual populations due to their lack of  
56 intrinsic variability and presumed scarce potential to buffer environmental challenges [8]. At  
57 an ecological timescale, migrational, behavioral, or physiological adjustments may facilitate  
58 the exposed individuals and populations to cope with the environmental change for a while  
59 [9, 10]. In an evolutionary context, there is increasing evidence of evolutionary change  
60 without standing genetic variation by epigenetic factors in asexual populations, including  
61 asexual aphids [11, 12]. From a classical point of view, though, an increased rate of  
62 environmental change would be difficult, at least in principle, for asexual populations to  
63 evolve adaptive responses quickly enough [2, 8]. Thus, rapid adaptation would be unlikely  
64 or at least unexpected, leaving the extinction of affected populations as a possible  
65 evolutionary outcome (but see [3]).

66 On the one hand, asexual reproduction has large immediate rewards. One of the  
67 most conspicuous advantages is that asexual individuals can allocate more energy and time  
68 to reproduction [13 – 15]. Consequently, asexual populations can show a clear demographic  
69 profit compared to their sexual counterparts [13 – 15]. When it comes to biological invasions,

70 asexuality can confer advantages to efficiently invade and monopolize new territories  
71 because only one individual is enough to multiply and start a new population [16]. Indeed,  
72 asexual lineages arriving and dominating extensive novel areas have been repeatedly  
73 reported [17 – 20].

74 On the other hand, in contrast, it has been suggested that the energetic and  
75 demographic advantages conferred by asexuality may be offset by the low resilience and  
76 evolvability of asexual populations [21 – 23]. Thus, many species that have moved away  
77 from strict sexual reproduction, adopting complex life cycles that include asexuality or strict  
78 clonality, could lose much of their ability to cope with environmental change, making them  
79 highly vulnerable to ecological challenges [14, 24, 25]. Consequently, the ecological (and  
80 evolutionary) persistence of an asexual population (or lineage) facing environmental change  
81 can be jeopardized by this lack of genetic diversity.

82 An extreme example of asexual populations with a high degree of geographic  
83 expansion is the case of aphids (Hemiptera: Aphididae). Aphids are sap-sucking insects  
84 characterized by a complex life cycle that alternates sexual and asexual reproduction [26,  
85 27]. However, they frequently lose the sexual phase, reproducing by obligate  
86 parthenogenesis (*i.e.*, clonal reproduction), especially in novel temperate environments [28,  
87 29, 30]. Thus, aphids are excellent invaders if environmental conditions are suitable due to  
88 their rapid demographic growth. Interestingly, some aphid clonal lineages may prevail over  
89 others in wide invaded ranges due to intraspecific exclusion and are commonly referred to  
90 as "superclones" [31]. Superclones can expand their geographical ranges significantly by  
91 means of phenotypic plasticity or, in some cases, with the assistance of bacterial secondary  
92 endosymbionts (see [32]). However, while superclones are common and their arrival,  
93 establishment, and expansion are well-documented (*e.g.*, [23, 33]), the factors that promote  
94 their persistence or exclusion in a given territory are poorly understood (but see Peccoud *et*  
95 *al.* [23]).

96 Aphid populations inhabiting central Chile represent an ideal system to study the  
97 response and persistence of an asexual population facing environmental change after local  
98 aphid populations are generally characterized by the predominance of a single or few  
99 superclones, regardless of the aphid species [23, 33 – 36]. On the one hand, despite the  
100 natural geographic barriers of the country [37], several aphid invasion processes have been  
101 reported in this range, which has increased in frequency given the growth in trade practices  
102 [38]. On the other hand, this extended territory is experiencing an extreme change in its  
103 abiotic environmental conditions, which would enable the study of population changes in  
104 real time. Indeed, temperatures have shown an increased tendency over the last decade,  
105 with summer heat wave events more common due to the pervasive effects of climate change  
106 [39 – 44].

107 In this context, the case of the pea aphid, *Acyrtosiphon pisum* (Harris), is  
108 remarkably interesting: (i) A genetic survey performed between 2005-2007 in central Chile  
109 [23] showed the predominance of an obligate asexual multilocus genotype (MLG) in alfalfa  
110 (*Medicago sativa*) crops, namely Ap2, inhabiting a ~600 Km latitudinal range (from 35 to  
111 40°S). (ii) For the same species, Sepúlveda *et al.* [45] found that, within this geographic  
112 range, *A. pisum* specimens collected on the same crop showed differences in the secondary  
113 symbionts they carried. These findings suggest the coexistence of different clonal lineages,  
114 considering that the main transmission mechanism for the secondary symbionts in aphids is  
115 maternal inheritance and that the pea aphid population of central Chile has shown to be  
116 strictly asexual [23, 46]. (iii) In a more recent work, Martel *et al.* [47] found not only one but  
117 two different MLGs in the same latitudinal range (APG2 and APG3) but in quite different  
118 proportions (~4% and ~96%, respectively). However, aphid MLGs were not comparable with  
119 the ones found by Peccoud *et al.* [23] because of some distinctive microsatellite markers  
120 and a different capillary sequencer used for this work (see Materials and Methods section in  
121 Martel *et al.* [47]).

122           Considering this background and the current process of environmental change due  
123 to global change across central Chile, the main objective of the present work was to evaluate  
124 the response of the clonal population of *A. pisum* inhabiting central Chile after a decade of  
125 facing extreme thermal conditions and the risk of intraspecific invasive events. We compared  
126 the current two lineages of *A. pisum* populations on alfalfa with the dominant lineage found  
127 by Peccoud *et al.* [23] using the same microsatellite markers and protocol. Considering that  
128 among the main environmental changes in central Chile over the last decade are the rise in  
129 mean summer temperatures and the increased prevalence and magnitude of summer heat  
130 waves [39 – 44], we hypothesized that the unbalanced proportions of the two MLG recorded  
131 by Martel *et al.* [47] could be attributable to differences in thermal response between  
132 lineages. Additionally, we assessed the presence/absence and the identity of the secondary  
133 endosymbionts in these two lineages, considering that some secondary endosymbionts can  
134 protect their host aphids from acute heat events [48, 49].

## 137 **2. MATERIALS AND METHODS**

### 138 *Sampling and laboratory rearing*

139 In a previous study [47], *A. pisum* individuals were sampled from alfalfa fields in the spring  
140 and summer of 2017-2018 in central Chile, the same region previously surveyed by Peccoud  
141 *et al.* [23] in 2006-2007 (latitude 35 to 40 °S, see [47]). This study showed that there were  
142 only two MLGs in this area (named APG2 and APG3 in [47]), with 96% of the genotyped  
143 individuals belonging to APG3 and the remaining 4% to APG2. Clonal lineages were  
144 established in the laboratory from these two MLG by rearing them separately on broad bean  
145 plants (*Vicia faba* L.) inside 4 L transparent plastic buckets, kept in a climatic chamber at  
146 20°C ( $\pm$  0.5°C) with a long photoperiod (LD = 16:8) to ensure their sustained parthenogenetic

147 reproduction [46, 50]. Five aphids from each lineage were taken before performing the  
148 experimental procedures on the thermal response (see below), and their DNA was extracted  
149 to confirm their genetic profile.

150

### 151 *Genetic profiles and secondary symbionts assessment*

152 Following Peccoud *et al.* [23], we used fifteen highly polymorphic genetic markers  
153 (microsatellites) to identify MLGs, including the seven original markers from [23] and eight  
154 additional markers from Nouhaud *et al.* [51] (Table 1). We extracted total genomic aphid  
155 DNA by the “salting-out” method, using proteinase-K digestion and precipitation by ethanol  
156 [52]. Then, the DNA was resuspended in ultrapure water and set to a concentration of 30  
157 ng/mL. Each locus was amplified separately with fluorescent-labeled primers following the  
158 same methodology performed by Peccoud *et al.* [23]. The electrophoresis of amplified  
159 fragments was completed using the same capillary sequencer ABI PRISM 3130xl (Applied  
160 Biosystems, U.S.A.), and the same laboratory set-up. MLG characterization was made after  
161 the automatic allele calls given by the software GeneMapper (version 3.5 and 3.7, Applied  
162 Corp., U.S.A.) and the visual checking of the allele sizes.

163 Besides carrying the obligate endosymbiont bacteria *Buchnera aphidicola*, aphids  
164 can host a range of facultative endosymbionts that can produce diverse phenotypic effects  
165 on the aphid host, including heat tolerance [32, 49]. Hence, we screened five facultative  
166 bacterial symbionts commonly reported in *A. pisum* by randomly selecting pea aphid  
167 samples (n = 5) from each clonal lineage. Aliquots of DNA extracted from live samples of  
168 APG2 and APG3 lineages were used to amplify diagnostic PCR fragments for *Spiroplasma*  
169 (1500 bp), *Serratia symbiotica* (1140 bp), *Regiella insecticola* (840 bp), *Rickettsia sp.* (600  
170 bp), *Hamiltonella defensa* (480 bp), and *B. aphidicola* (270 bp), the obligate aphid symbiont,  
171 used as control [42, 43]. The amplification steps were an initial DNA denaturation at 94 °C  
172 for 5 min, 30 cycles of 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 1 min, and a final



173 extension step at 72 °C for 5 min. Amplicons were separated through electrophoresis in  
174 1.5% agarose gels, using a GeneRuler 100 bp Plus DNA Ladder (Thermo Fisher Scientific,  
175 Waltham, Massachusetts, USA). The presence of each facultative endosymbiont was  
176 visually identified according to the size of the diagnostic amplicon in base pairs (bp) obtained  
177 from the DNA of each aphid [46].

178

### 179 *Temperature and heat-shock effects on life-history traits*

180 To evaluate the performance of individuals from APG2 and APG3 lineages following  
181 exposure to short and extreme heat events, we used an already-established protocol used  
182 by Montlor *et al.* [48] and Russell and Moran [53] on the pea aphid using two extreme  
183 temperatures registered in central Chile (*i.e.*, 38 and 42 °C; [36-41]). In brief, 40 to 60 age-  
184 synchronized aphid nymphs of each lineage were separately kept on *V. faba* seedlings in  
185 three separated climatized chambers at 20°C ( $\pm$  0.5°C, LD = 16:8). After 30 min of  
186 acclimation, trials started by increasing the temperature of two chambers to 38°C and 42°C  
187 (heat-shock treatments, heating rate = 0.15 and 0.18 °C/min respectively) in the two first  
188 chambers respectively, while the third chamber was kept with no temperature change (20°C  
189 constant, control). The temperature rose for 2 h for each treatment until the trial temperature  
190 was reached. Once reached, this temperature was maintained for 4 h and then lowered to  
191 20°C in the next 2 h (total heat challenge time = 8.5 h). After the trials, individuals were  
192 returned to their original acclimation temperature (20°C  $\pm$  0.5°C, LD = 16:8) for 48 additional  
193 hours to allow for recovery and avoid over-manipulation. Subsequently, after checking that  
194 all the plants were well, surviving aphids were counted to obtain the proportion of alive/dead  
195 individuals and randomly transferred to new *V. faba* seedlings until they were reproductive  
196 adults. Furthermore, we replicated the control treatment at constant rearing temperatures of  
197 15 and 25°C ( $n \geq 6$  individuals per temperature).

198 Comparisons of reproductive performance were made by using intrinsic growth rates  
199 ( $r_m$ ), calculated as a derivation from the Euler-Lotka equation, where  $r_m = \ln(R_0)/t$  (see Birch  
200 [54]). Fecundity ( $R_0$ ) of the survivor nymphs was measured as the number of nymphs  
201 produced by each focal individual aphid in a lapse of ten days since the birth of its first nymph  
202 since this period generally comprises 95% of the total progeny of an aphid [55]. The  
203 developmental time ( $t$ ), in turn, was measured as the number of days elapsed from the focal  
204 individual's birth to the birth of its first nymph.  $R_0$  and  $t$  were recorded individually for the  
205 maximum number of survivors after the trials for each MLG and heat challenge combination.  
206 To summarize, five experimental treatments were used: three constant acclimation  
207 temperatures (15, 20, and 25 °C) and two heat-shock temperatures (38 and 42 °C), totaling  
208 154 individual measures of  $r_m$ , a consistent number given the clonal nature of these aphid  
209 lineages (see [56]).

210

### 211 *Data analysis*

212 Since the MLGs used in this study came from the same region previously surveyed by  
213 Peccoud *et al.* [23], a Neighbor-Joining (NJ) tree of MLGs was built with the MLGs found in  
214 this study and those found by Peccoud *et al.* [23] using the seven original microsatellite  
215 markers to make both studies comparable. NJ tree was constructed based on a distance  
216 matrix of allele shared distances ( $D_{AS}$ ) with bootstrapping of 1000 replicates and computed  
217 by the software Populations v1.2.31 [57].

218 To analyze and compare the intrinsic growth rate of each lineage within their thermal  
219 niche, we fitted a linear model after a visual diagnostic of the data using constant acclimation  
220 temperature and MLG as independent variables and intrinsic growth rate as dependent  
221 variable. Then, predicted values of  $r_m$  were used to show how detected differences in  $r_m$   
222 between genotypes could be translated into an ecologically relevant scenario. We assessed  
223 how daily population growth and cumulative population size are comparatively predicted to

224 change as a function of mean diurnal temperatures (08:00 – 18:00 h). This time range was  
225 selected to capture better the influence of heat-temperature episodes recorded in the field.  
226 First, we obtained temperature data at hourly intervals for the Quinta Normal weather station  
227 (33°25'S, 70°42'W) in Santiago for the years 2018, 2019, and 2020, sourced from the  
228 Chilean Agricultural Research Institute (<https://agrometeorologia.cl/>, data downloaded on 12  
229 May 2022). We chose this locality because the effects of thermal change are representative  
230 of the predominant climate in central Chile [44]. This location shows a marked seasonality,  
231 and it is a centered spot considering the wide latitudinal distribution of the pea aphid along  
232 central Chile. Temperature was converted into Kelvin degrees (adding 273°) and then  
233 returned to the Celsius scale to avoid possible confounding effects of below-zero  
234 temperatures. Then, predicted  $r_m$  based on these thermal conditions were separately  
235 converted into daily population growth ( $\lambda$ ) for APG2 and APG3, and subsequently into  
236 cumulative population size as  $N(t) = \prod \Delta N$  over a three-year period (1096 days in total,  
237 considering one leap year). For simplicity, calculations assume that initial population sizes  
238 were identical for both genotypes and negative  $r_m$  predicted from the linear regressions were  
239 set to zero (*i.e.*, aphids are assumed not to die due to cold, which is likely realistic during the  
240 growth period of aphid populations). On the other hand, to estimate how different MLG  
241 responded to the heat-shock treatment, we performed a multiple regression including  $r_m$  as  
242 the dependent variable, genotype as a categorical factor, and heat-shock temperature as a  
243 covariable.

244

245

### 246 **3. RESULTS**

247 The results of the genetic analysis showed that MLGs Ap2 reported by Peccoud *et al.* [23]  
248 and APG2 reported by Martel *et al.* [45] shared the same microsatellite profile at the fifteen  
249 evaluated loci (Table 1). On the other hand, the genetic profile of APG3 showed allele

250 differences at fourteen out of fifteen loci compared to Ap2 and APG2 (Table 1). Thus,  
251 considering (i) the genetic identity between Ap2 and APG2 at the fifteen loci evaluated and  
252 (ii) that both MLGs are obligate parthenogenetic lineages (see Peccoud *et al.* [23] and Martel  
253 *et al.* [47]), our results suggest that Ap2 and APG2 belong to the same clonal lineage (Ap2  
254 = APG2, Fig. 1A, in blue), hereafter APG2, which was widespread in central Chile before  
255 2007 (Fig. 1B, in blue). On the other hand, the NJ tree representing shared allele distances  
256 suggests that APG3 is a novel lineage genetically distant from those previously reported by  
257 Peccoud *et al.* [21] (Fig. 1A, in green). Interestingly, the frequency of APG2 has dramatically  
258 changed between the two studies in central Chile. While Peccoud *et al.* [21] showed that  
259 98% of the individuals sampled on alfalfa belonged to APG2 (n = 100 out of 102), the  
260 proportion of APG2 dropped to ~4% a decade later. The APG3 variant constituted ~96% of  
261 the sampled populations (n = 6 and 137 respectively, out of 143), appearing as a newly  
262 emerged genotype of *A. pisum* in central Chile (Martel *et al.* [47]). Additionally, none of the  
263 five facultative symbionts studied were detected in APG2 (n = 5), as opposed to APG3 (n =  
264 5) specimens, all of them showing a stable coinfection with *H. defensa* and *Rickettsia sp.*  
265 along the experimental procedures (Table 1).

266 Aphid's reproductive performance at different acclimation temperatures differed  
267 between genotypes, with APG2 and APG3 displaying higher  $r_m$  at cold and warm  
268 temperatures, respectively. This impact was significantly different between MLGs (Fig. 2A)  
269 based on the interaction between genotype and acclimation temperature in the linear model  
270 ( $F_{1,68} = 28,34$ ,  $p = 1.23 \times 10^{-6}$ ). The predicted  $r_m$  values based on the linear regressions for  
271 each MLG were used to test for a clonal lineage turnover in the field using a real temperature  
272 dataset (Fig. 3A). In this regard, Fig. 3B depicts the difference in daily population growth of  
273 APG3 (in green) relative to APG2 (in blue) when temperatures are warm. While the opposite  
274 trend is observed when temperatures are cool, the simulation shows that the population size  
275 of APG3 is expected to increase disproportionately compared to APG2 under warm periods

276 (Fig. 3C). Moreover, these differences are expected to become more pronounced under  
277 heat stress conditions. Following the 38°C and 42°C heat shock laboratory assays, both  
278 MLG lineages exhibited a notorious detrimental effect on relative survival, developmental  
279 time ( $t$ ), offspring number ( $R_0$ ), and, consequently, on intrinsic growth rate ( $r_m$ ) (Table 2).  
280 However, heat tolerance varied significantly between MLGs as APG3 better copes with heat  
281 stress (Fig. 2B), as evidence by the significant interaction between MGL and heat-shock  
282 temperature in our multiple regression ( $F_{1,118} = 6.72$ ,  $p = 0.011$ ).

283

#### 284 **4. DISCUSSION**

285 In this study, we report a dramatic turnover between two strictly asexual genotypes of the  
286 pea aphid in central Chile over a decade, presumably following a new and recent invasion  
287 event (Fig. 1A and 1B). Our results suggest that this turnover might result from the  
288 contrasting reproductive performance displayed by both APG2 (original) and APG3  
289 (putatively invasive) lineages experiencing the changing thermal conditions of this region,  
290 characterized in part by increasingly common heat-shock events in the last decade (Fig. 2  
291 and Fig. 3).

292 High genetic diversity is commonly considered advantageous for natural populations  
293 because of a higher adaptative capacity than populations with low genetic variation [14, 25,  
294 58]. Indeed, hereditary variability combined with sex can allow for rapid evolutionary change  
295 and adaptation to changing ecological conditions [59]. It is thought that this variability could  
296 promote invasions, as genetically diverse invaders with an enhanced evolutionary potential  
297 may successfully establish and spread in the novel range [60, 61]. In contrast, variability in  
298 invaded populations could favor resilience to invasions, reducing their extinction risk [22].  
299 Consequently, local asexual populations can be severely threatened by invaders because  
300 of low phenotypic variation in some traits (both at the intra- and inter-specific level),  
301 potentially leading to competitive exclusion [62]. Thus, the lack of genetic diversity of the

302 pea aphid in central Chile due to its strictly asexual reproductive mode in this area [23, 47]  
303 could explain the rapid change in the genetic structure of this population in a decade by the  
304 replacement of the original superclone (APG2) by a genetically distant one (APG3; Fig. 1).  
305 Importantly, APG3 is quite divergent from the MLGs found by Peccoud *et al.* [23] on any  
306 host plant including alfalfa, and even from any genotype reported by Martel *et al.* [47]  
307 inhabiting the southern region of Chile which could have served as a source of genetic  
308 variation (Fig. 1A and [47]). This suggests either the introduction of a new lineage between  
309 2007 and 2017 or the clonal amplification of a previously present MLG but at such a low  
310 frequency that it was not detectable in previous samples.

311         Despite the differences in reproductive performance between both lineages following  
312 the heat challenge (Table 2 and Fig. 2), other changes than thermal conditions can also be  
313 involved in the superclone turnover reported here. Multiple factors, such as differences  
314 between the two clones in their ability to fend off natural enemies, cope with inter-specific  
315 competition, or resist insecticide applications, can be relevant to explain this shift in clonal  
316 frequency [63]. However, we have no evidence for rapid changes in the guilds of natural  
317 enemies in central Chile [64, 65] nor the type of insecticide treatment [66]. In contrast, there  
318 is well-supported evidence for climate change during the period of turn, including extreme  
319 summer temperatures in central Chile in recent years, reaching unprecedented records  
320 above 42°C in 2017 and 2019 [39 – 44]. Thus, we propose that the pattern observed in the  
321 field (Fig. 1B) is more related to different physiological responses to the thermal environment  
322 between APG2 and APG3 (Table 2 and Fig. 2). For instance, APG2 has a greater  
323 reproductive output at low temperature (*i.e.*, 15 °C) compared to APG3. In contrast, its  
324 survival and reproductive output facing acute warming episodes is lower (Figure 2). Thus,  
325 APG2 would start reproducing earlier at low temperatures but would be replaced by APG3  
326 after heat stress (Fig. 3). In this sense, the heat challenge approach remains to some extent  
327 realistic as it estimates the impact of high temperatures in fitness, which mirrors adequately

328 the conditions encountered by aphid populations in nature (Fig. 3). Importantly, these  
329 differences in ‘fitness’ and population growth between MGL are expected to be exacerbated  
330 at higher environmental temperatures (*i.e.*, through time with global warming or across  
331 warmer localities). Having said that, specific mechanisms giving thermal advantage to APG3  
332 over APG2 after acute extreme heat events remain unknown.

333 Interestingly, it has been reported that facultative bacterial endosymbionts can confer  
334 ecological advantages to the aphid host, such as thermal tolerance by mechanisms aiding  
335 the recovery of the obligate symbiont, *B. aphidicola*, after heat stress [32, 48, 67], leading,  
336 for example, to seasonal dynamics of secondary symbionts prevalence [67 - 70]. For  
337 instance, in populations of the grain aphid *Sitobion avenae*, the presence of the  
338 endosymbiont *S. symbiotica* might partly explain recent changes in the composition of  
339 superclones observed in Chile (Sepúlveda *et al.*, submitted). However, *S. symbiotica* was  
340 not detected in APG3, and neither *H. defensa* nor *Rickettsia sp.*, the bacterial  
341 endosymbionts detected in APG3 (Table 1), have been unequivocally demonstrated to  
342 confer protection to heat stress in previous studies (but see [53]), although this is  
343 undoubtedly something worth exploring in future research. In this sense, one limitation of  
344 our study was the impossibility of effectively disinfecting facultative symbionts from APG3  
345 individuals and, conversely, infecting APG2 with them, something that is not always  
346 successful due to biological or technical limitations [see 71]. Disentangling the respective  
347 effects of symbiont and aphid genotypes would have provided valuable insights into the  
348 mechanism responsible for the observed turnover.

349 Overall, our study suggests that a rapid turnover of *A. pisum* superclone composition  
350 occurred in central Chile, likely driven by warmer temperatures, emphasizing that extreme  
351 environmental changes can trigger different responses across genotypes that could  
352 ultimately end up in competitive exclusion [72]. Notably, the lack of detectable genetic  
353 variability in the previous and more recent populations of *A. pisum* strongly supports some

354 form of competitive displacement, either triggered by environmental changes alone or by the  
355 introduction of a new genotype. Short but extreme thermal events, concurrently with  
356 increased mean temperatures, could have contributed to the emergence of APG3 due to its  
357 increased thermal endurance (Table 2 and Fig. 2), leading to a competitive advantage  
358 against APG2 for the same ecological niche (Fig. 3). This phenomenon is comparable to  
359 some extent with that reported by Brévault *et al.* [73] for the cotton aphid *A. gossypii*, in  
360 which only one of the two overrepresented genotypes was prevalent (>90%) in insecticide  
361 sprayed cotton producing regions of the west and central Africa, despite the equivalent or  
362 even higher reproductive performance of a second clone on non-sprayed fields. Overall, our  
363 results show that asexual populations can thrive over large areas under favorable conditions  
364 on ecological timescales and may even be able to adapt to environmental changes [11, 12,  
365 74, 75]. However, asexuality and clonal reproduction suffer from a lack of adaptability on an  
366 evolutionary scale, potentially leading to the extinction of entire asexual populations and the  
367 collapse of their genetic heritage.

368

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372

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380 **Availability of data and material** The data and the R script were deposited in DRYAD  
381 digital repository under the reference number <https://doi.org/10.5061/dryad.j0zpc86m6>.

382 **Author's contributions** SIM, JCS, CCF, ELR, and FB conceived the ideas and designed  
383 methodology; SIM, CAZ, JCS, and FM collected the data; SIM, CAZ, NR, DAS, JCS, CCF  
384 and ELR analyzed the data; SIM wrote the first draft and led the writing of the manuscript.  
385 All authors contributed to subsequent drafts and gave their final approval for publication.

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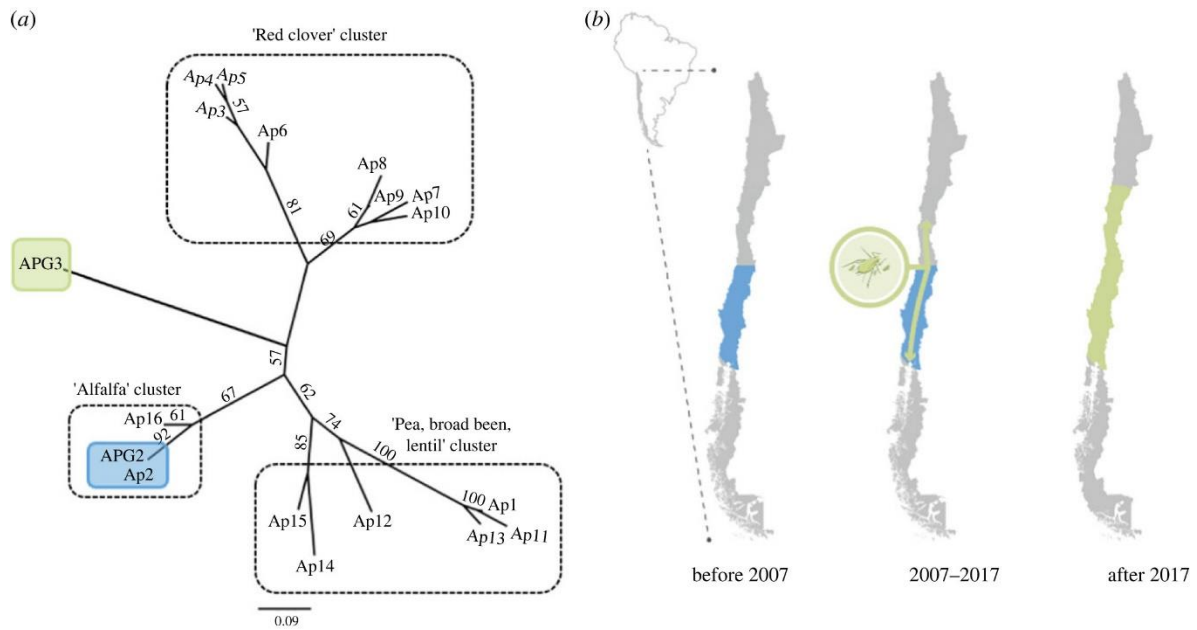
591 **Table 1.** Allele combinations at fifteen microsatellite loci, including seven reported by Peccoud *et al.* [21] (in bold) and eight reported by  
592 Nouhaud *et al.* [49], for the clonal lineages of the pea aphid found in central Chile. The composition of facultative bacterial symbionts  
593 detected in alive individuals from APG2 and APG3 are also shown.

Lineage (MLG)	Study	Locus ID															Facultative symbionts
		AIA09M	AIB07M	AIB08M	AIB12M	ApF08M	ApH08M	ApH10M	111775	114782	117294	118775	118777	126104	127526	128001	
Ap2	Peccoud <i>et al.</i> 2008	308/310	117/153	276/284	312/318	163/175	246/248	196/198	275/302	301/303	372/376	219/219	345/355	282/287	406/416	203/207	-
APG2	Martel <i>et al.</i> 2020	308/310	117/153	276/284	312/318	163/175	246/248	196/198	275/302	301/303	372/376	219/219	345/355	282/287	406/416	203/207	None
APG3	Martel <i>et al.</i> 2020	306/308	117/133	258/272	304/330	165/171	248/266	184/194	275/281	295/301	378/382	219/219	343/351	281/292	408/410	198/200	<i>H. defensa</i> / <i>Rickettsia</i>

594 **Table 2.** Raw mean data for relative survival, generational time in days ( $t$ ), fecundity to the 10<sup>th</sup> day ( $R_0$ ), and intrinsic growth rate ( $r_m$ )  
 595 for aphids of the APG2 and APG3 lineages following the temperature shock assays. Values are shown as mean  $\pm$  SE.

596

MLG	Replicates	Temperature (°C)	Relative survival	$t$ (days)	$R_0$ (# nymphs)	$r_m$ (days <sup>-1</sup> )
APG2	6	15	1	14.8 $\pm$ 0.2	104.3 $\pm$ 1.7	0.314 $\pm$ 0.004
	20	20	1	9.7 $\pm$ 0.1	96.4 $\pm$ 1.2	0.474 $\pm$ 0.006
	10	25	1	6.5 $\pm$ 0.2	69.5 $\pm$ 3.6	0.655 $\pm$ 0.021
	22	38	0.77	10.5 $\pm$ 0.4	45.0 $\pm$ 8.6	0.283 $\pm$ 0.042
	16	42	0.38	11.8 $\pm$ 0.5	4.2 $\pm$ 3.2	0.043 $\pm$ 0.024
APG3	6	15	1	15.7 $\pm$ 0.8	52.0 $\pm$ 4.5	0.255 $\pm$ 0.018
	20	20	1	9.2 $\pm$ 0.1	93.0 $\pm$ 2.1	0.491 $\pm$ 0.006
	10	25	1	6.1 $\pm$ 0.1	90.1 $\pm$ 4.1	0.738 $\pm$ 0.013
	25	38	1	10.5 $\pm$ 0.2	66.1 $\pm$ 5.0	0.399 $\pm$ 0.015
	19	42	0.84	11.8 $\pm$ 0.6	22.0 $\pm$ 5.0	0.223 $\pm$ 0.034

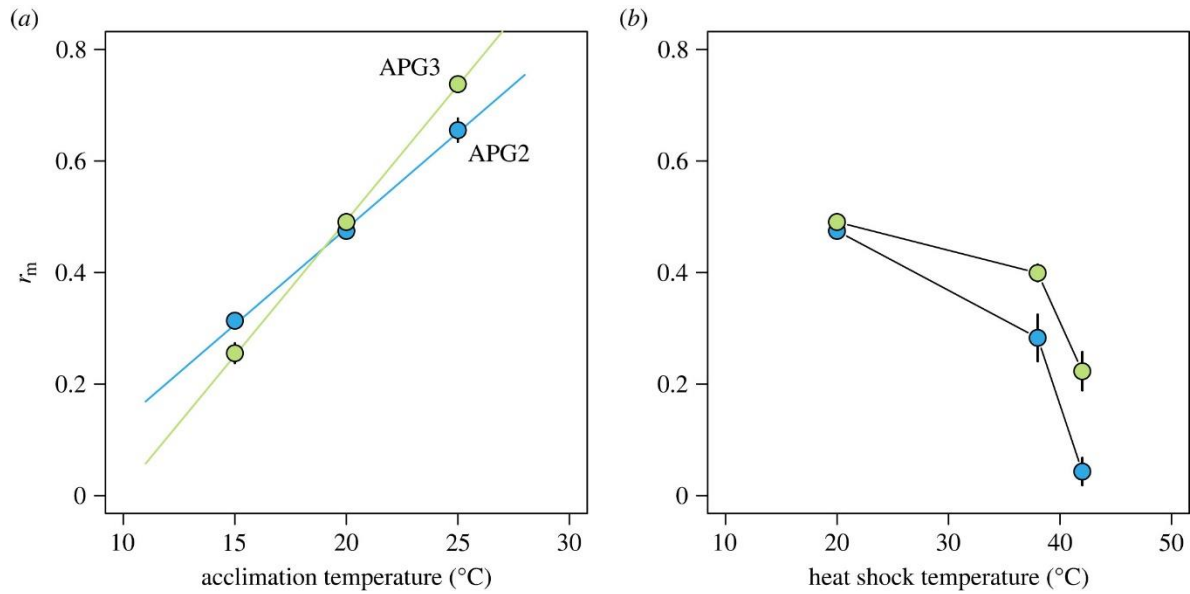


597

598 **Figure 1.** (A) Neighbor-Joining tree using allele shared distances for seven microsatellite loci [23]  
 599 combining the MLGs studied in this work (APG2 and APG3) and the 16 MLGs (Ap1 – Ap16) for all  
 600 host species found in central Chile by Peccoud *et al.* [23]. Dashed boxes indicate MLG clusters relying  
 601 on genetic differences and related to host species [23]. Colors indicate the original (APG2, blue) and  
 602 the putative invasive (APG3, green) alfalfa lineages. Numbers along branches represent the  
 603 bootstrap percentage of the supporting nodes (shown > 50 %). (B) Hypothetical model for the invasion  
 604 of APG3 in central Chile.

605

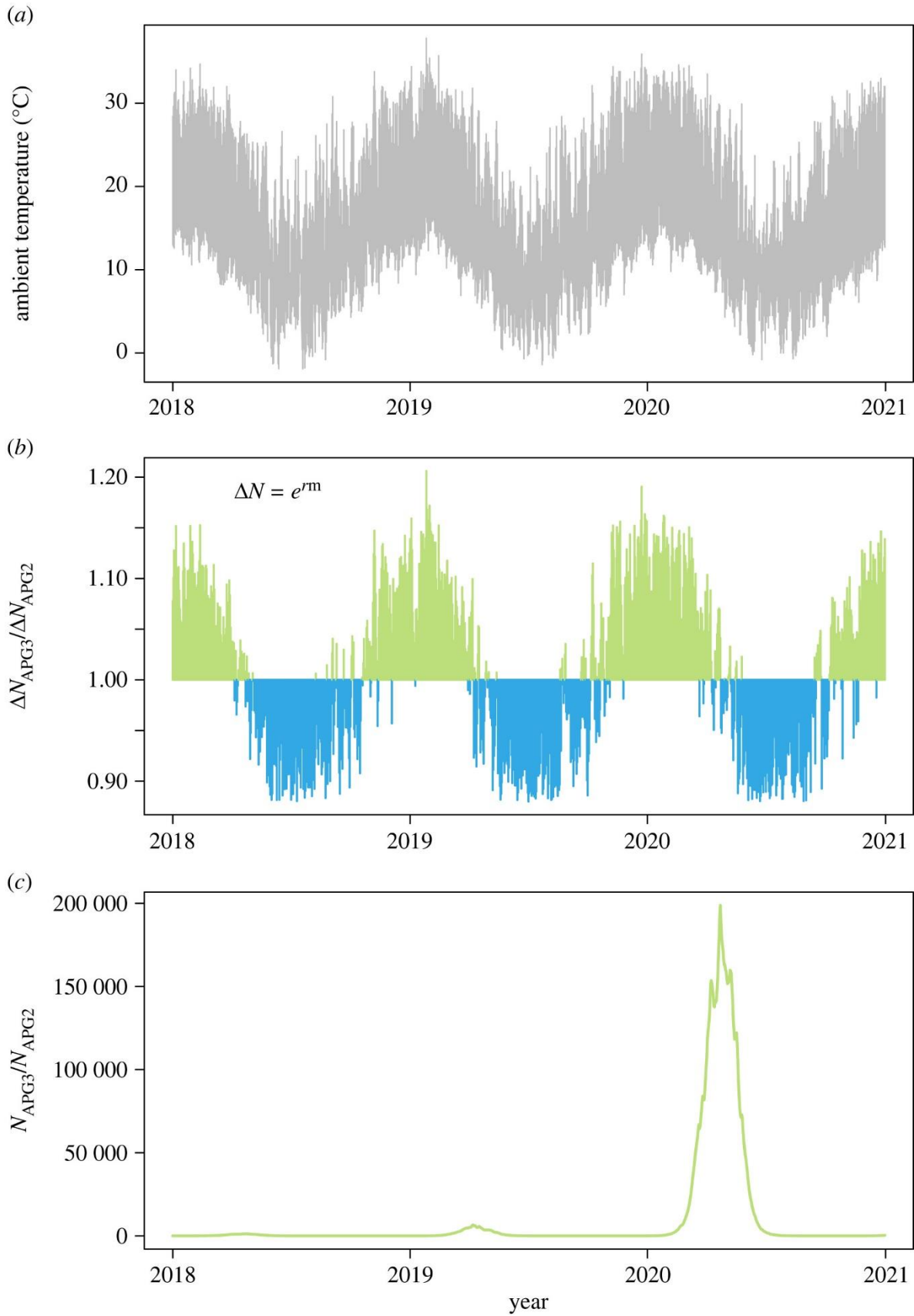
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607

608 **Figure 2.** Mean intrinsic growth rate ( $r_m$ ) for both MLG lineages used in this study (APG3 in green  
 609 and APG2 in blue) by experimental settings: (A) constant acclimation (15, 20, and 25 °C), and (B)  
 610 heat-shock (38 and 42 °C). Values are mean  $\pm$  SE. In panel A, lines represent linear regressions  
 611 performed separately for each genotype ( $R^2 > 0.89$ ), used to simulate comparatively the population  
 612 growth of each MLG under field conditions (see Figure 3). Panel B shows the decline in  $r_m$  after  
 613 experimental heat-shock procedures at 38 and 42° (see Materials and Methods section).

614



616 **Figure 3.** Comparative population responses to ambient temperature between APG3 (in green) and  
617 APG2 (in blue) using  $r_m$  predictions from linear regressions (Fig. 2) and real field temperatures from  
618 central Chile (see Materials and Methods section). (A) Hourly ambient temperatures of Quinta Normal  
619 (33°25'S, 70°42'W) for a three-year period. (B) Population growth ratio (daily) between APG3 and  
620 APG2. (C) Cumulative population size ratio between APG3 and APG2.