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Running title: Extensive pea aphid superclone turnover

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

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ABSTRACT

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

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

1. INTRODUCTION

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

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

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

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

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

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

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

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

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

2. MATERIALS AND METHODS

Sampling and laboratory rearing

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

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

Genetic profiles and secondary symbionts assessment

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

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

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

Temperature and heat-shock effects on life-history traits

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

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

Data analysis

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

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

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

3. RESULTS

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

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

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

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

4. DISCUSSION

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

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

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

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

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

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

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

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

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Competing interests The authors declare no conflict of interest.

Ethics approval Ethics approval was not required for this study according to the institutional normative.

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

Author's contributions SIM, JCS, CCF, ELR, and FB conceived the ideas and designed methodology; SIM, CAZ, JCS, and FM collected the data; SIM, CAZ, NR, DAS, JCS, CCF and ELR analyzed the data; SIM wrote the first draft and led the writing of the manuscript. 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/ Rickettsia</i>

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

MLG	Replicates	Temperature (°C)	Relative survival	t (days)	R_0 (# nymphs)	r_m (days ⁻¹)
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

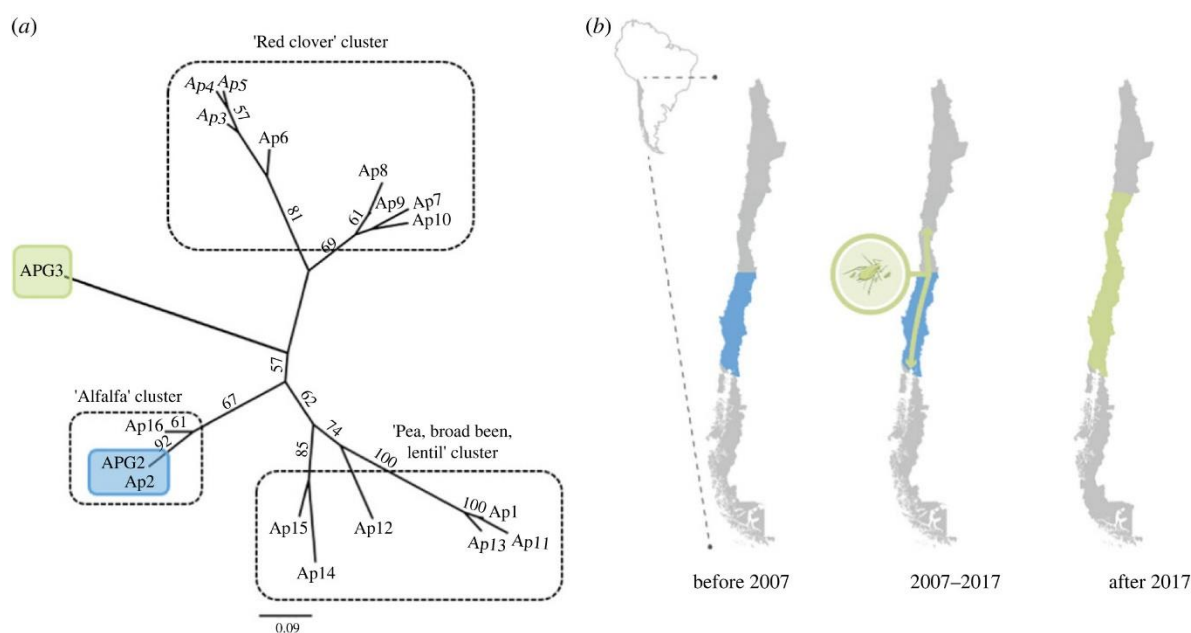


Figure 1. (A) Neighbor-Joining tree using allele shared distances for seven microsatellite loci [23] combining the MLGs studied in this work (APG2 and APG3) and the 16 MLGs (Ap1 – Ap16) for all host species found in central Chile by Peccoud *et al.* [23]. Dashed boxes indicate MLG clusters relying on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

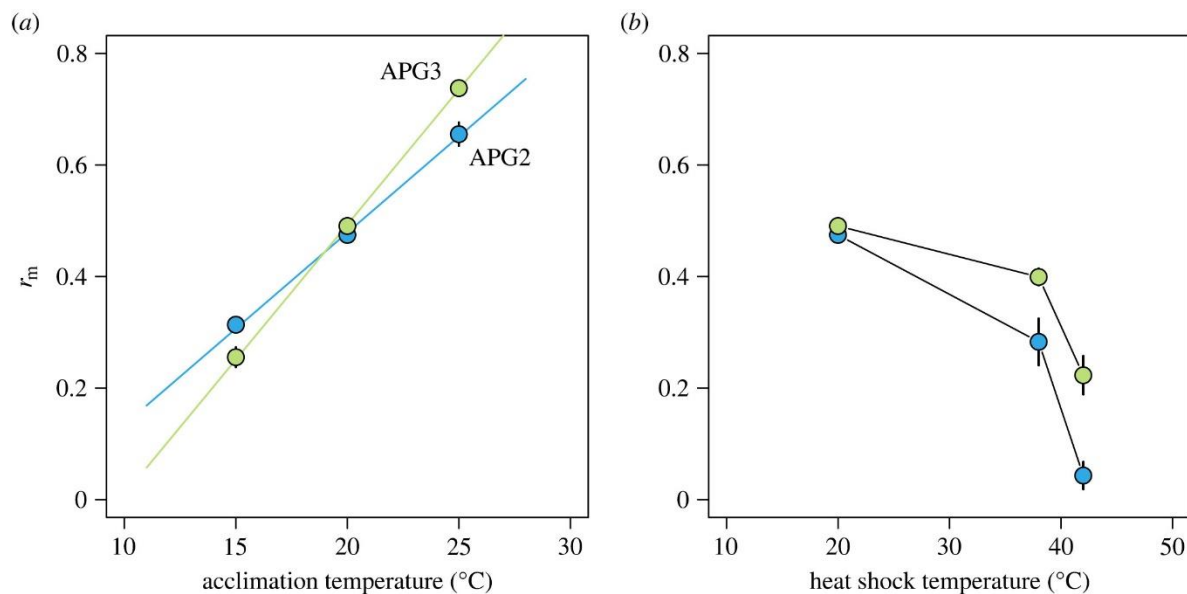


Figure 2. Mean intrinsic growth rate (r_m) for both MLG lineages used in this study (APG3 in green and APG2 in blue) by experimental settings: (A) constant acclimation (15, 20, and 25 °C), and (B) heat-shock (38 and 42 °C). Values are mean \pm SE. In panel A, lines represent linear regressions performed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

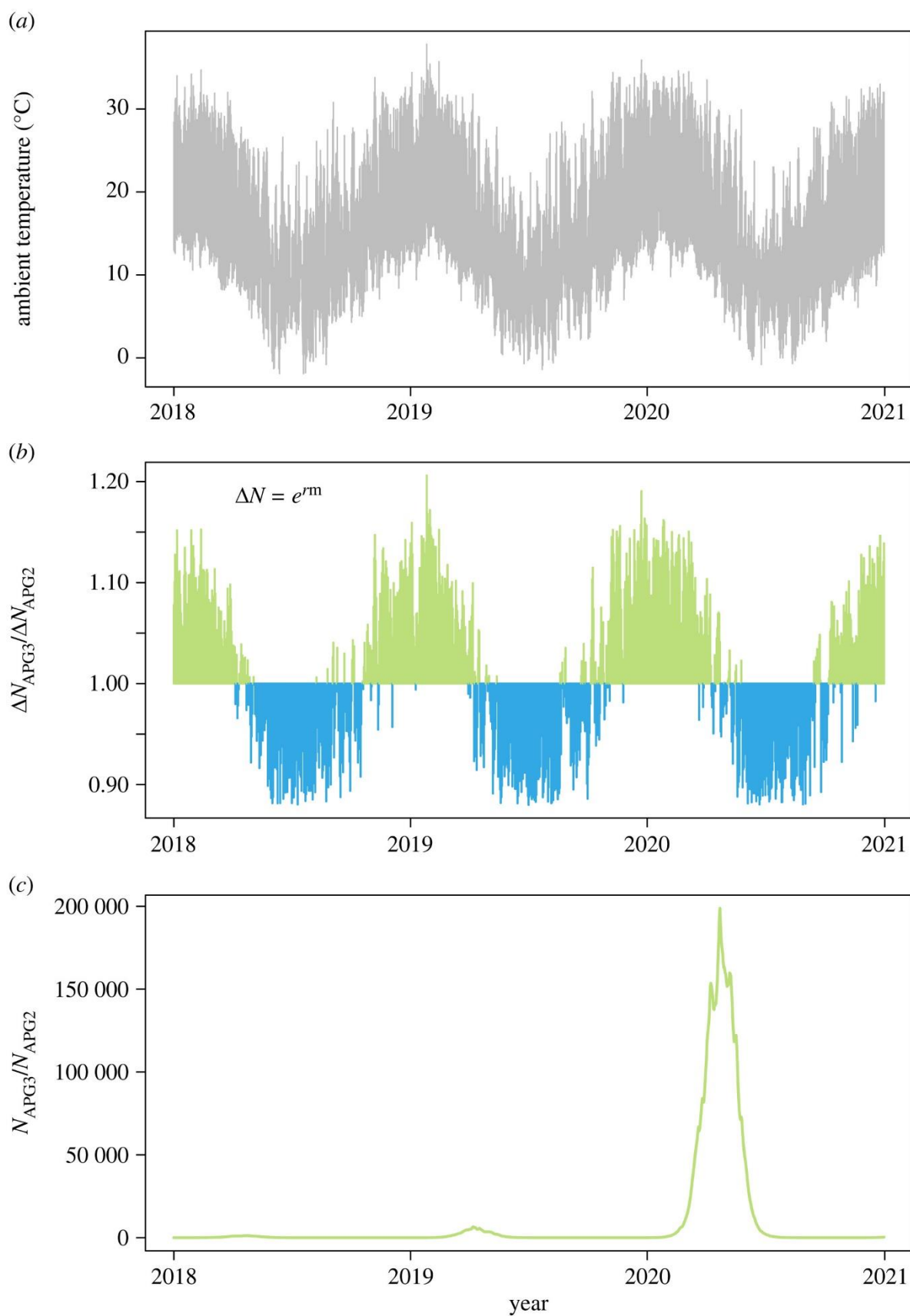


Figure 3. Comparative population responses to ambient temperature between APG3 (in green) and APG2 (in blue) using r_m predictions from linear regressions (Fig. 2) and real field temperatures from central Chile (see Materials and Methods section). (A) Hourly ambient temperatures of Quinta Normal (33°25'S,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