Anthropogenic fire drives the evolution of seed traits

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Fire is a major disturbance affecting ecosystems worldwide. Phylogenetic studies have shown that the evolution of seed persistence (fire resistance) is associated with fire frequency or severity. However, the existence of specific seed traits resulting from natural selection mediated by fire remains a key question in plant evolution. We evaluated the role of fire in the evolution of seed traits from a microevolutionary perspective, using as a study system a native forb from the Chilean matorral, where fire is a novel, anthropogenic disturbance. We show that anthropogenic fires are shaping the evolution of seed traits such as pubescence and shape. Among-population variation in seed pubescence, shape, and pericarp thickness was strongly associated with fire frequency, and within a population, fire selected those plants with more pubescent seeds, thicker pericarps, and less rounded seeds. Seed pubescence and shape were shown to be heritable traits. Our findings provide insights into the understanding of the evolution of seed traits in fire-prone environments and demonstrate that human-made fires can be driving evolutionary changes in plant species from ecosystems where fires do not occur naturally.

fire ecology | Mediterranean ecosystems | microevolution | seed germination

Fire is a global-scale disturbance that has influenced the structure of natural plant communities over evolutionary time (1–3), driving plant diversification (4). In most Mediterranean ecosystems (the Mediterranean Basin, California, South Africa, and Australia), fire is a natural and predictable selective pressure that is associated with summer thunderstorms. Several plant species in these ecosystems have developed adaptive responses to fire such as resprouting, serotiny, and seed germination by fire-related cues (heat shock, smoke, or charred wood) (5). Fire-stimulated germination and fire resistance of seeds are considered to be adaptive features because they allow plant establishment after fire, that is, under low competition and high resource availability (5, 6). Macroevolutionary studies indicate that fire has driven the diversification of plant species with seed persistence (fire-resistant or fire-stimulated seed banks) in Mediterranean ecosystems, particularly in plant communities where fire has been more frequent or intense (7). At an ecological scale, some studies have correlated the variation of seed persistence with fire regime among species or populations (8–10). Nevertheless, natural selection operates within populations, and we have little knowledge on how fire acts on the variability of seed traits among individuals to drive evolutionary changes in natural populations.

Trait evolution by natural selection will occur if there is (*i*) variation in the trait within the population, (*ii*) a significant relationship between the trait and relative fitness, and (*iii*) heritability of the trait (11). Furthermore, a relationship between the frequency (or intensity) of the selective pressure and trait expression across populations is expected; in addition, the stronger the natural selection, the lower the variability of the trait in the populations (12). Because natural selection is easier to be detected when selective pressures are strong and novel in the system (13), naturally fire-free ecosystems that are currently burned by human-made fires provide an outstanding opportunity to explore the evolutionary ecology of seed traits in relation to

fire. This is the case of the Mediterranean-type vegetation of central Chile (the matorral). Unlike other Mediterranean ecosystems, lightning-ignited fires are extremely rare in the Chilean matorral. It has thus been hypothesized that fire has not been a relevant factor in the evolution of seed persistence in this ecosystem (14, 15). Keeley et al. (16) recently suggested that lightning-ignited fires may have been relatively common in central Chile in the Miocene, thus explaining some fire-adaptive traits such as lignotubers of some woody species, but that the virtual absence of fire in the region during the last few million years probably accounts for the lack of fire-dependent reproduction in matorral flora.

On the other hand, anthropogenic fires have been common since the Spanish colonization in the mid-16th century, and their frequency has increased exponentially since the 19th century (17). Therefore, the reintroduction of fire, this time as an anthropogenic disturbance, might have selected favorable responses in native plant species, particularly in annuals or short-lived species (18, 19). Seed germination is stimulated by heat or smoke in a few matorral species (14, 18), thus indicating the occurrence of seed persistence in the native flora. Seed persistence is commonly associated with hard, thick, and impermeable seed coats (5), and heat and smoke derived from fire can cause testa scarification and break seed dormancy (5, 20). Fire-stimulated germination is more frequently found in small-seeded species (10, 21), and small, rounded seeds tend to form persistent seed banks, as they are easily buried (22). Seed pubescence might contribute to seed thermoregulation (23) and could protect buried seeds from fire damage, but this has not been tested or considered previously.

Helenium aromaticum (Hook.) L. H. Bailey (Asteraceae) is a native annual herb distributed along the Mediterranean zone of Chile (24). The dispersal unit of this species is the achene, which includes the seed plus the testa and the pericarp, but we will refer to it as the seed. Seeds are coniform, with a short pappus, and moderately pubescent (24). It has been shown recently that seed bank emergence of *H. aromaticum* increases after fire (25), and seeds are viable after heat-shock and smoke treatments (26). Furthermore, we have observed monospecific stands of *H. aromaticum* after fires in some matorral communities (Fig. S1). We used this annual forb as a model study to test the hypothesis that fire can drive the evolution of seed traits. Specifically, we hypothesized that anthropogenic fire has exerted a selective pressure on seed traits of *H. aromaticum*, so that (*i*) plants from populations located in habitats with high fire frequency would have smaller, more rounded, and pubescent seeds with thicker coverings (testa and pericarp) compared with plants from areas

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with low fire frequency; (*ii*) the variability of seed traits in *H. aromaticum* populations would decrease with fire frequency; (*iii*) the probability of germination after fire within a population would be associated with the expression of the evaluated seed traits; and (*iv*) fire-selected seed traits would be heritable in *H. aromaticum* populations.

To test these hypotheses, we located 10 *H. aromaticum* populations across central Chile (Fig. S2) and used dendrochronological techniques to estimate fire frequency and site productivity from co-occurring *Acacia caven* (Fabaceae) trees to correlate it with seed traits (mean and coefficient of variation). Afterward, we chose an *H. aromaticum* population with intermediate fire frequency [Lampa (LP); Fig. S2] and carried out a fire-selection experiment to estimate the relationship between the probability of seed germination and seed traits (adaptive hypothesis). Finally, we estimated the heritability of those seed traits that were shown to be under selection by fire, analyzing the linear relationship between the expression of those traits in maternal plants and their progeny.

Results

Seed pubescence, seed shape (width:length ratio), and pericarp thickness were positively and significantly correlated with the fire frequency of *H. aromaticum* habitats (Fig. 1 *A–C*). Furthermore, these traits were not associated with site productivity (pubescence: $r^2 < 0.001$, $r = 0.007$, $P = 0.98$; shape: $r^2 = 0.18$, $r = 0.43$,

$P = 0.22$; pericarp: $r^2 = 0.07$, $r = 0.27$, $P = 0.45$; linear regression, $n = 10$), indicating that fire would be more relevant in explaining their regional variation compared with other environmental factors related to site quality (e.g., water and nutrient availability). In contrast, seed size and testa thickness were not correlated with fire frequency (size: $r^2 = 0.16$, $r = 0.40$, $P = 0.25$; testa: $r^2 = 0.11$, $r = 0.34$, $P = 0.34$; linear regression, $n = 10$), and testa thickness was positively associated with site productivity ($r^2 = 0.65$, $r = 0.80$, $P < 0.01$; linear regression, $n = 10$).

We did not find a significant negative relationship between fire frequency and the coefficient of variation for any of the evaluated seed traits (pubescence: $r^2 = 0.125$, $r = -0.35$, $P = 0.31$; shape: $r^2 = 0.11$, $r = -0.33$, $P = 0.35$; pericarp: $r^2 = 0.001$, $r = 0.04$, $P = 0.91$; size: $r^2 = 0.01$, $r = -0.09$, $P = 0.80$; testa: $r^2 = 0.04$, $r = -0.20$, $P = 0.58$; linear regression, $n = 10$).

Germination after experimental fire was higher in hairier seeds, in those seeds with thicker pericarps (positive directional selection: $\beta_{pubescence} = 0.69 \pm 0.31$, $P = 0.03$; $\beta_{pericarp} = 0.89 \pm 0.35$, $P = 0.01$; multiple regression), and in less rounded seeds, that is, with lower values of width:length ratio (negative directional selection: $\beta_{shape} = -0.68 \pm 0.31$, $P = 0.03$; multiple regression). In contrast, seed size and testa thickness were not associated with the probability of germination ($\beta_{testa} = -0.19 \pm 0.34$, $P = 0.58$; $\beta_{size} = -0.42 \pm 0.23$,
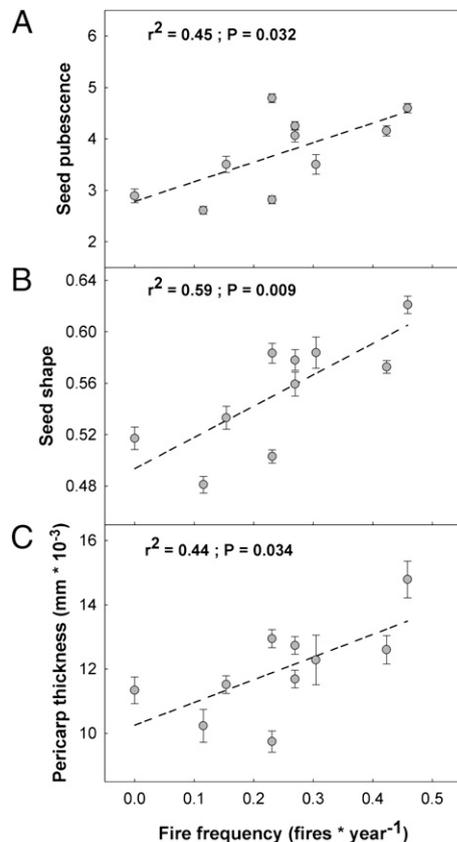

Fig. 1. Correlation between seed traits and fire frequency across *H. aromaticum* populations in the Chilean matorral. (*A*) Seed pubescence (according to six categories of increasing pubescence). (*B*) Seed shape (width:length ratio). (*C*) Pericarp thickness. Dots represent the mean value of each population, and error intervals are SE ($n = 25$ plants, except for pericarp thickness, where $n = 10$ plants). Dashed lines correspond to the fitted models. $P < 0.05$ indicates that the slope is significantly different from zero (linear regression, $n = 10$ populations).
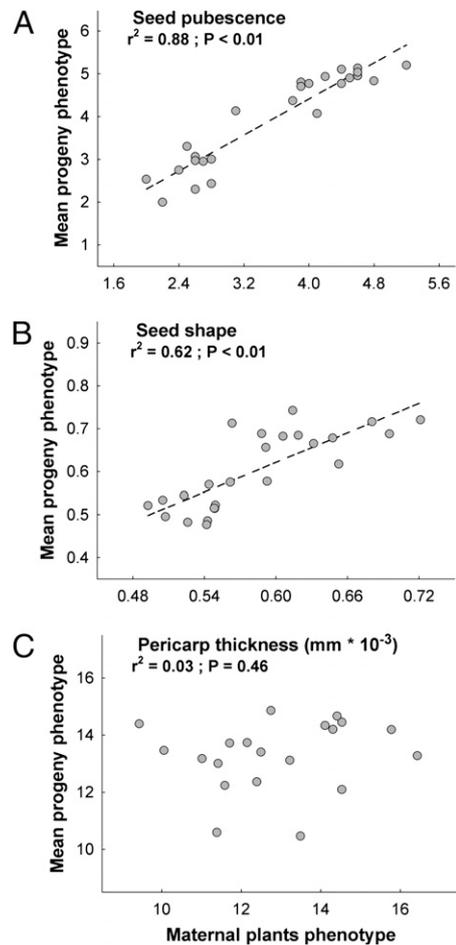

Fig. 2. Heritability of fire-selected seed traits in a population of *H. aromaticum*. Relationship between the maternal phenotype and the offspring phenotype (mean of three full sibs). (*A*) Seed pubescence (according to six categories of increasing pubescence). (*B*) Seed shape (width:length ratio). (*C*) Pericarp thickness. $P < 0.05$ indicates that the slope is significantly different from zero (linear regression, $n = 25$ individuals, except for pericarp thickness, where $n = 20$).

P = 0.07; multiple regression). Seed pubescence and shape were heritable traits; there was a significant positive relationship between the expression of these traits in maternal plants and their progeny (Fig. 2 *A* and *B*), whereas pericarp thickness was not inherited by the progeny (Fig. 2*C*).

Discussion

Although seed persistence in Mediterranean plants is generally recognized as an adaptation to fire (5, 6, 10), some authors consider that its evolution might have been caused by other selective factors, such as drought or herbivory (27). This controversy arises from the practical difficulty of demonstrating whether the evolution of a particular trait resulted from the exclusive effect of fire in the past, because in nature multiple factors interact as selective pressures on plant populations (6). We herein tested experimentally an adaptive hypothesis for several seed traits that could be involved in fire resistance and studied the patterns of seed trait variation across populations. We thus show that fire is a selective pressure that shapes seed traits, predominating over the influence of other environmental factors subsumed into site productivity. Specifically, the association between fire frequency and the interpopulation variation in seed pubescence, seed shape, and pericarp thickness indicates that anthropogenic fire has driven the evolution of these traits in *H. aromaticum*. The selection experiment showed that seed pubescence, seed shape, and pericarp thickness have an adaptive value, that is, their expression enhances plant fitness. This indicates that human-made fire is changing the phenotypic frequency distribution in natural populations by selecting more pubescent, thicker-coated, and less rounded seeds (Fig. S3 *A–D*). Furthermore, seed pubescence and shape are heritable, and therefore conditions are given by natural selection in *H. aromaticum* seed traits for evolution to occur. Interestingly, over 30 y ago, *H. aromaticum* seeds were described as "moderately pubescent, mainly on the ribs" (24), but we found a gradient of pubescence across populations, with seeds from frequently burned sites being densely pubescent. Indeed, there was a dramatic increase (35%) in seed pubescence from the site with no fires (San Carlos; Fig. S3*A*) to the site with 11 fires [Los Aromos, cultivated area (LAC); Fig. S3*B*]. This could be because the actual historical range of fire frequency is probably wider than that shown by the fire scar chronology (Table S1). Nevertheless, natural selection by fire seemingly was not strong enough to reduce the within-population variation of seed traits. This suggests that other selective factors, such as seed predation or drought, or other evolutionary processes, such as gene flow, might somewhat counteract the effects of fire on seed trait variation.

The functional role of seed hairiness with regard to fire is yet to be revealed. It might confer protection to the shallowly buried seeds against fire damage by increasing insulation. Regardless of the underlying mechanism of protection against fire, the whole body of evidence presented here demonstrates that anthropogenic fire has been involved in the evolution of seed pubescence in *H. aromaticum*. Seed shape is also heritable, but in this case the within-population trend, with less rounded seeds being selected by fire, opposes the regional pattern, where a positive relationship between seed "roundness" and fire frequency was found. It is likely that rounded seeds are more common in frequently burned sites because they are easily buried (21) and thus better protected from fire than elongated seeds. It remains to be explained why in the selection experiment less rounded seeds were proven advantageous. Experimental fire intensity could have been insufficient to break seed dormancy in rounded phenotypes. The patterns of selection on seed shape could be complex and modulated by the interplay between soil texture (related to burial probability), seed dormancy, and fire intensity.

Our results demonstrate that anthropogenic fire has shaped adaptive traits in *H. aromaticum* populations, thus challenging the widespread assumption that native plant species from the Chilean matorral are not adapted to fire because of its historical absence as a natural disturbance (13, 14). A recent study reported an increase in the species richness of native annuals in several matorral communities following fires (18), which suggests that fire adaptations might be rather common in matorral species. Natural selection can be extremely rapid (28), particularly in short-lived species and when selective pressures are strong and recent (12). We have shown that phenotypic selection mediated by fire is now occurring in plants from naturally fire-free ecosystems, and this may have significant consequences on the evolutionary trajectory of plant populations. Under the current global scenario in which fire frequency and intensity are increasing due to climate change and human activities (29, 30), the role of anthropogenic fire as a selective agent in ecosystems worldwide is probably being underestimated.

Materials and Methods

Species. *H. aromaticum* is an annual Asteraceae, 20–60 cm tall, branched at the top of the stem, with apical globose yellow heads 7–12 cm in diameter (24). Seeds (achenes) are 1.4–1.7 mm long, 0.6–0.8 mm wide, ribbed, with variable pubescence and a 1.3–1.9 mm long pappus (24). The species generally inhabits dry hills at 500–1,000 m elevation, flowering in December and setting fruit from January to March. Seeds are dispersed at short distances by gravity and wind.

Fire Frequency and Site Productivity. To assure a wide gradient of fire frequency along the 10 *H. aromaticum* populations, we preselected sampling areas likely to include sites with high and low fire frequency according to maps of fire history produced by CONAF (Forestry National Corporation). Once a specific *H. aromaticum* population was found within a given area, additional information on the fire history of the site was collected by interviewing land managers (Table S1). Then, for each site finally selected (Fig. S2), a fire chronology was constructed from dated fire scars of five to eight individuals of the tree species *A. caven* (Fabaceae) (Fig. S1). Cross-sections of *A. caven* were surfaced using an electric hand planer. The ring structure and cellular detail were revealed using progressively finer grit sandpaper (220–600 grit). Visual cross-dating was made following the techniques described by Stokes and Smiley (31). COFECHA software (32) was used to ensure the accuracy of both relative and absolute dating of the samples. We estimated the fire frequency of each site as the number of fire events observed from 1982 to 2008, except for LP and LAC populations, where the oldest dates were in 1985 and 1986, respectively (Fig. S4). Furthermore, to ascribe more confidently the regional variation in seed traits to fire frequency, we estimated site productivity as the potential confounding factor affecting seed trait expression. Considering the same time period, we calculated the mean annual ring increment (MAI = $\sum$ ring$_i$ width/i, where i = 26 y) in five *A. caven* cross-sections per site, and the average MAI (n = 5) was used as a proxy for site productivity. Only one of the evaluated seed traits, testa thickness, showed a significant relationship with site productivity (see above). Because testa thickness was not associated with fire frequency nor under selection by fire (see above), this result does not affect the general conclusions of the study. Furthermore, we found no significant association between fire frequency and site productivity (r^2 = 0.28, r = 0.08, P = 0.431; linear regression, n = 10), indicating that the variation of fire frequency is not linked to an environmental gradient.

Seed Traits. During February 2009, we collected mature seeds from 25 plants of each of the 10 *H. aromaticum* populations. Seeds were always collected from plants growing in open, sunny microhabitats, thus avoiding differences in microclimatic conditions among populations. Five seeds per plant were randomly selected and digital pictures were taken at 10× magnification. Images were analyzed with SigmaScan Pro (Systat Software) to measure seed surface (size) and width:length ratio (shape). Additionally, seed pubescence was visually estimated under a light microscope (10× magnification), assigning a category of pubescence from 1 to 6 (Fig. S5). When seed pubescence seemed intermediate between two categories, we added 0.5 to the lowest value. We used mean pubescence (n = 5 seeds) as a response variable in our analyses, so the variable "seed pubescence" was continuous.

We randomly selected three seeds from 10 plants per population to measure the thickness of seed coverings. To this end, we performed hand-made cuts in the middle part of the seeds. Cross-sections were fine enough to distinguish the anatomy of the seeds under a light microscope at 20× magnification. They were photographed and the digital images were

processed as described above to measure testa and pericarp thickness. *H. aromaticum* seeds are covered by a unicellular testa that is attached to the pericarp (Fig. S3 C and D). Under the light microscope, the pericarp appears as a dark, dense layer, which is covered by cube-shaped epidermal cells (Fig. S3 C and D). Epidermal cells were easily broken and lost after cutting the seeds, so they were not included in the measures. Testa and pericarp were measured between the ribs, because they were folded and irregularly shaped in the rib area.

Selection Experiment. We exposed seeds taken from a natural population to experimental fire under controlled conditions. We chose a population with an intermediate fire frequency (LP; Fig. S2) and collected a large number of mature seeds from 80 plant individuals (families) in the field. For each of these 80 families, 100 seeds were used in the selection experiment and 5 seeds were reserved to make trait measurements as described above. These 100 seeds were placed in a clay pot and covered with a 1-cm stratum of sterile, dry sand. We chose a 1-cm depth because previous experimental fires have shown that all seeds are killed when they are left on the soil surface and that soil temperature at 2.5-cm depth (35 °C) is too low to produce effects on seeds (25). All pots ($n = 80$) were topped with dry litter (taken from a matorral community) and then fire was ignited with a blowtorch. To eliminate viable seeds from other species, which may bias germination probability estimations, litter was previously sifted and heated (110 °C; 20 min). We used 12 g of litter per pot to simulate the natural fuel load in *H. aromaticum* habitats (1.06 kg·m^{-2}) (25). Using copper-constantan thermocouples, we measured the temperature during the experiment in eight samples at 1 cm below the sand stratum. The mean maximum temperature was 91.6 °C (±6.5; SE) and the residence time over 50 °C was 15 min (±2.7; SE). Burning grassland litter produced a great amount of smoke, lasting 8–10 min. After the experiment, pots—including their whole content of sand, seeds, and charred litter—were placed in a germination chamber (12 h light at 20 °C; 12 h dark at 10 °C). All pots were watered and checked daily for *H. aromaticum* seedling emergence for 31 d. The probability of germination of each individual was used as a measure of fitness for the phenotypic selection analysis. Afterward, we tested the viability on nongerminated seeds (1% 2,3,5-triphenyltetrazolium chloride in phosphate buffer, pH 7.3, 24 h in darkness). No statistical relationship was detected between seed viability and seed traits ($\beta_{pubescence} = -0.14 \pm 3.54$, $P = 0.91$; $\beta_{pericarp} = 7.04 \pm 4.04$, $P = 0.08$; $\beta_{testa} = -4.02 \pm 4.12$, $P = 0.33$; $\beta_{shape} = 0.38 \pm 3.34$, $P = 0.91$; $\beta_{size} = -1.07 \pm 2.39$, $P = 0.65$; multiple regression), indicating that phenotypic selection targets seed traits that influence germination success.

Heritability. We estimated the heritability of fire-selected seed traits by analyzing the relationship between maternal plants and their progeny for such traits (parent–offspring regression method) (33). We focused on the same population of the selection experiment (LP). Seed traits of 25 maternal plants were measured as described above. Some of these seeds were then used to obtain the progeny ($n = 3$ full sibs per maternal family). Seeds were germinated and seedlings were planted in pots with a 2:3 sand:field soil mixture. All pots were watered every 2 d. Plants were grown in a common environment in a greenhouse until flowering. Heads were protected from pollinators with a fine, transparent mesh until seed maturation. Five inbred seeds from each F2 plant were measured as described above.

Statistical Analyses. We used linear regressions to assess the association of the mean and the coefficient of variation (SD per mean) of seed traits across *H. aromaticum* populations with fire frequency and/or site productivity. To test the adaptive hypothesis in the LP population, we used a multiple-regression approach (34). Selection gradients were calculated by regressing the probability of seed germination on standardized traits (mean = 0, SD = 1). We used generalized linear models and *t* tests (for quasibinomial distribution) to assess the significance of the estimated parameters. Linear selection gradients (β_i) assess the magnitude of directional selection acting on the trait's mean, quadratic gradients (γ_i) measure selection acting on the variance of the trait and hence assess the form (stabilizing or disruptive) of the selection function, and correlational gradients (γ_{ij}) reveal particular combinations of trait states that are selected together (34). Before performing the selection analyses, we ruled out colinearity between traits by means of Pearson correlations (0.29 < r < 0.74; Table S2) because it could cause spurious results (35). We did not find evidence for correlated selection (Table S3). Only one significant (negative) quadratic gradient was detected (Table S3), but it was evident that this resulted from a change in the slope of the fitness function rather than from the occurrence of a maximum fitness value and hence it did not reflect true stabilizing selection (Fig. S6) (35). Heritability of selected traits was estimated by regressing the family mean of the progeny ($n = 3$) over the maternal plant phenotype for each seed trait (33). A trait was considered heritable when the regression slope was positive and significantly greater than zero ($P < 0.05$; linear regression).

All statistical analyses were performed using R 2.8.0 software (36).

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