

RESEARCH PAPER

Masting behaviour in a Mediterranean pine tree alters seed predator selection on reproductive output

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ABSTRACT

- Context-dependency in species interactions is widespread and can produce concomitant patterns of context-dependent selection. Masting (synchronous production of large seed crops at irregular intervals by a plant population) has been shown to reduce seed predation through satiation (reduction in rates of seed predation with increasing seed cone output) and thus represents an important source of context-dependency in plant-animal interactions. However, the evolutionary consequences of such dynamics are not well understood.
- Here we describe masting behaviour in a Mediterranean model pine species (*Pinus pinaster*) and present a test of the effects of masting on selection by seed predators on reproductive output. We predicted that masting, by enhancing seed predator satiation, could in turn strengthen positive selection by seed predators for larger cone output. For this we collected six-year data (spanning one mast year and five non-mast years) on seed cone production and seed cone predation rates in a forest genetic trial composed by 116 *P. pinaster* genotypes.
- Following our prediction, we found stronger seed predator satiation during the masting year, which in turn led to stronger seed predator selection for increased cone production relative to non-masting years.
- These findings provide evidence that masting can alter the evolutionary outcome of plant-seed predator interactions. More broadly, our findings highlight that changes in consumer responses to resource abundance represent a widespread mechanism for predicting and understanding context dependency in plant-consumer evolutionary dynamics.

INTRODUCTION

Over the last half century, a great deal of work has been put forward to understanding the ecological and evolutionary consequences of species interactions (Abrahamson 1989; Price *et al.* 1991; Thompson 1999). Within the context of plant-herbivore interactions, there is ample evidence that herbivores act as selective agents by influencing plant defensive (Rausher & Simms 1989; Valverde *et al.* 2001; Stinchcombe & Rausher 2002; Agrawal 2005), reproductive (Leimu *et al.* 2002; Kolb & Ehrlén 2010; Abdala-Roberts & Mooney 2014) and ecophysiological traits (Salgado-Luarte & Gianoli 2012) in plant populations. However, recent investigations have also demonstrated that herbivore selection on plants is in many cases context-dependent, as both biotic and abiotic factors can alter the outcomes of these interactions (Johnson & Stinchcombe 2007; Kolb & Ehrlén 2010; Maron *et al.* 2014). For example, in the case of biotic factors, several studies have observed that herbivore selection on plants is influenced by third-party species,

such as natural enemies (Abdala-Roberts *et al.* 2014) and/or mutualists, such as pollinators (*e.g.* Herrera *et al.* 2002; Gómez 2003; Strauss & Irwin 2004; Sletvold *et al.* 2015). Likewise, in the case of abiotic environment, factors such as soil resource availability (Abdala-Roberts *et al.* 2014) and light availability (Kolb & Ehrlén 2010; Salgado-Luarte & Gianoli 2012) have been shown to influence herbivore selection on plant traits. Although these studies have made valuable progress in understanding how the biotic and abiotic forcing influences the evolution of plant-herbivore interactions, we still lack a full understanding of the ecological mechanisms driving changes in herbivore selection (Abdala-Roberts *et al.* 2014; Abdala-Roberts & Mooney 2015).

Most consumers respond to resources in a density-dependent fashion and, as a result, interaction strength and therefore selection should depend on resource density (Abdala-Roberts & Mooney 2015). Nevertheless, the evolutionary consequences of density-dependent interactions are relatively poorly understood. Within the context of plant-herbivore interactions,

consumption is frequently density-dependent, and thus any factor that alters resource density will in turn influence the rate of consumption and thus potentially also influence herbivore selection on plant traits (Abdala-Roberts & Mooney 2014; Abdala-Roberts *et al.* 2014). Although consumer functional responses have traditionally received a great deal of attention from the point of view of population ecology (Holling 1959; Abrams 1982), their evolutionary significance has received far less empirical attention (for theoretical work see Abrams 2000).

One potentially important form of context dependency in consumer selective effects on plants may take place through changes in the timing and synchrony of reproductive events across individuals within a plant population. A prime example of this is mast seeding (also called 'masting'), which consists of the synchronous production of large seed crops at irregular time intervals by a population of iteroparous plant species (Silvertown 1980; Kelly 1994). Masting is present across a wide spectrum of plant taxonomic groups and terrestrial ecosystem types, and is particularly widespread among temperate trees (Silvertown 1980; Kelly 1994). One of the most widely accepted theories to explain masting behaviour is the predator satiation hypothesis (PSH), which proposes that super-abundant, synchronous and temporally variable seed production maximises seed escape through starvation of predators in most years, followed by satiation of reduced seed predator populations during occasional mast years (Janzen 1976; Silvertown 1980). The occurrence of seed predator satiation is dependent upon the type of functional response exhibited by seed predators; in particular, Type II and Type III functional responses (*sensu* Holling 1965), which describe negative density-dependent attack (*i.e.* seed predation rates decrease with increasing seed availability), are expected to lead to seed predator satiation (Fletcher *et al.* 2010). Type II responses describe a decelerating increase in seed consumption towards a maximum value of resource abundance (Holling 1959). The proportion of seed consumed by predators is thus highest at low levels of seed availability (Fig. S1). Type III responses describe positive density dependence at low resource densities, then an inflection point where attack is highest, and finally a switch to negative density dependence towards zero (Holling 1959). The proportion of seeds consumed by predators is highest at some intermediate level of seed availability and then, when the resource becomes less abundant, declines towards zero (Fig. S1). The measurement and characterisation of functional responses of seed predators to masting has received considerable attention, and at least two previous studies have demonstrated that negative density-dependent attack by seed predators is higher during masting events (Fletcher *et al.* 2010; Linhart *et al.* 2014). However, the evolutionary consequences of such dynamics have received little attention (but see Siepielski & Benkman 2008; Espelta *et al.* 2009). By altering the rate of predation (consumption rate relative to resource density), masting can also alter seed predator selection on plant reproductive traits.

Here, we present a novel test of the effects of masting behaviour on natural selection imposed by seed predators on plant reproductive traits. To do so, we collected 6-year data on seed cone production, spanning one mast year and five non-mast years, from a genetic trial composed by 116 unrelated maritime pine (*Pinus pinaster* Ait.) genotypes were replicated in ten blocks (N = 1160 trees). For each tree, we recorded seed cone

production and classified cones as damaged (*i.e.* attacked by vertebrate and invertebrate seed predators) or undamaged. First, we examined the functional response of seed predators to seed cone output at the individual tree level. Following the PSH, we hypothesised that the functional response would be a Type II or Type III negative density-dependent response where attack rates decrease with increasing cone output (*i.e.* predator satiation), leading to weaker attack rates in mast years. Second, we examined if, by altering the seed predator functional response, masting behaviour influenced the selective effects of seed predators on seed cone output. In particular, we evaluated whether negative density-dependent attack resulted in positive selection by seed predators on cone output. Although positive selection on cone output is expected because cone number is a component of fitness, we expected that density-dependent seed predator attack would strengthen this selection as genotypes with higher reproductive output suffer from lower attack rates, and this would enhance relative fitness of those genotypes in the presence of seed predation (Leimu *et al.* 2002; Abdala-Roberts *et al.* 2014). We hypothesised that selection exerted by seed predators to produce larger seed cone crops would be stronger during the mast year because masting enhances negative density dependence (decreasing attack and thus higher fitness with increasing cone output in the presence of seed predators). Overall, this study highlights that the alteration of consumer functional responses provides a widespread mechanism underlying changes in the evolutionary outcomes of species interactions and suggests a novel form of context dependency in such evolutionary dynamics (see also Bogdziewicz *et al.* 2016; Pesendorfer & Koenig 2016; Zwolak *et al.* 2016).

MATERIAL AND METHODS

Pine species and seed predators

Maritime pine (*P. pinaster*) is a fast-growing conifer native to the Mediterranean Basin and Atlantic coast of Europe, where it is widely distributed at low to moderate altitudes, mostly from sea level to 600 m a.s.l. (Richardson 1998). As in most pine species, mature female cones ('seed cones' hereafter) are located on the upper branches and require two growing seasons after pollination to mature, reaching their full size by the autumn of the second year. During the following spring–summer, with the first events of high temperatures, seed cones open and release small seeds with large wings and high nutritional content. As in other Mediterranean pines, mast seeding events are common in a high proportion of maritime pine populations (X. Moreira, personal observation). At our study site, seed cones of maritime pine are attacked by seed-eating specialist insects such as the European cone weevil *Pissodes validirostris* (Gyll.) (Coleoptera: Curculionidae), the cone moths *Dioryctria mendacella* (Stgr.) (Lepidoptera: Pyralidae) and *Gravitar mata margarotana* (Hein) (Lepidoptera: Tortricidae), as well as generalist vertebrates such as the passerine bird *Sitta europea* L. (Passeriformes: Sittidae) and the common squirrel (*Sciurus vulgaris* L., Rodentia: Scuridae). Both insect and vertebrate seed predators consume the seeds of 2-year-old cones, and attacked cones abort and do not open (Linhart *et al.* 2014). Seed predation is the main reason for abortion of mature seed cones (>90% of cases; E. Merlo, personal observation).

Field site, experimental design and measurements

We carried out a 6-year field survey of a 15-year-old *P. pinaster* forest genetic trial with 116 unrelated genotypes clonally replicated in ten blocks ($n = 1160$ trees, belonging to 116 pine genotypes \times 10 blocks). In the first week of December over 6 years (from 1998 to 2004, except 2002 due to technical problems), we monitored each individual tree for total seed cone production by inspecting the crown and counting the total number of new mature seed cones produced. We classified seed cones as cones with no signs of seed predator damage ('healthy seed cones' hereafter) and cones with external evidence of damage ('attacked seed cones' hereafter). In our study, the latter resulted finally in aborted cones in all cases. The forest genetic trial is located at Sergude (A Coruña, Galicia, NW Spain, 42.818261°N, 8.461284°W) and belongs to the Galician Tree Breeding Programme (Consellería do Medio Rural, Xunta de Galicia, for more details about this genetic trial see Appendix S1).

Data analyses

Classification of mast and non-mast years

Following previous studies, we defined mast-seeding years as years when seed production exceeds the mean seed production across all sampling years by a designated amount, measured in standard deviations (SD; LaMontagne & Boutin 2009; Linhart *et al.* 2014; Moreira *et al.* 2014). For this study, we calculated the population deviation from the long-term mean in SD for each year as $SD_i = (X_i - X_p) / (SD_p)$, where the SD_i and X_i are the SD and the mean for year i and X_p and SD_p are the SD and mean for the population across all 6 years. 'Mast years' were then defined as those where $SD_i \geq 1.0$, while 'non-mast years' were defined as those where $SD_i < 1.0$ (see LaMontagne & Boutin 2009; Linhart *et al.* 2014; Moreira *et al.* 2014). This approach identified one mast year (2004) and five non-mast years (1998, 1999, 2000, 2001, 2003; Fig. S2).

Functional response of seed predators

First, we determined if there was genetic variation for total seed cone production (total; *i.e.* healthy and attacked) and the proportion of attacked seed cones (calculated as the number of attacked cones divided by total cone production) across all 6 years, and separately for mast and non-mast years using general linear mixed models (PROC MIXED in SAS 9.4; Cary, NC, USA; Littell *et al.* 2006), where we included genotype as a fixed factor and block as a random factor. Data met the assumption of normality of residuals (using identity as link function).

Subsequently, we performed a logistic regression with a logit function using genotype-specific means (*i.e.* average value across plants from each genotype) across all 6 years to determine the type of functional response exhibited by seed predators (PROC GENMOD in SAS 9.4; Littell *et al.* 2006). For this, we evaluated the type of functional response by testing for the effects of the linear and quadratic term for total seed cones across all 6 years on the proportion of attacked seed cones (Trexler *et al.* 1988; Juliano 2001; Fletcher *et al.* 2010). Relative to other statistical techniques used to determine the shape or type of functional responses, the logistic regression approach described above produced the most accurate estimates of the true regions of density dependence (Trexler *et al.* 1988). A

Type I functional response is described by non-significant linear and quadratic coefficients of seed cones; a Type II response is indicated by a significant negative linear coefficient of seed cones; and a Type III response is indicated by a significant positive linear coefficient and a negative quadratic coefficient of cones (Trexler *et al.* 1988; Juliano 2001; Fletcher *et al.* 2010). Types II and III functional responses both support the predator satiation hypothesis because the proportion of seed consumed by predators decreases with increasing seed availability across at least some range of seed density (Holling 1959).

After assessing the overall type of functional response, we then tested whether masting influenced seed predator attack rates (*i.e.* if masting strengthened satiation) by testing for the effects of the linear and quadratic term for total seed cones, year type (mast *versus* non-mast year), and their interaction on the proportion of attacked seed cones with a logistic regression (binomial distribution and logit link) in PROC GENMOD (SAS 9.4) using genotype means. An interaction between year type and seed cone number indicates that the slope of the relationship between the proportion of attacked seed cones and seed cone number is influenced by year type (mast *versus* non-mast year). The reference condition for year type was a mast year, and therefore a positive value for the interaction parameter indicates a shallower slope (*i.e.* lower proportion of attacked seed cones per seed cone produced) and thus stronger satiation in mast than non-mast years. We did not find significant differences in the functional response of seed predators across non-mast years, confirming that the function of seed predator recruitment was relatively homogeneous across non-mast years (X. Moreira *et al.*, unpublished data). Therefore, data were pooled across non-mast years by using sums across this 5-year period.

Effect of masting on seed predator selection

We evaluated whether negative density-dependent attack resulted in selection by seed predators for larger cone output (Miller *et al.* 2008; Abdala-Roberts & Mooney 2014; Abdala-Roberts *et al.* 2014), and whether such selection was stronger during the mast than in the non-mast years. To test for such effects, first we calculated the relative fitness of each genotype separately for each year type (*i.e.* mast *versus* non-mast) under two scenarios of seed predator attack by computing seed cone output in the presence and absence of seed predation. For non-mast years we summed values across years. Under the scenario where seed predator effects were absent, relative fitness for each genotype was calculated by dividing the total number of seed cones (attacked + not attacked) for each tree by the mean total number of seed cones across all trees. Under the scenario where seed predator effects were present, relative fitness was calculated by dividing the number of healthy seed cones of each tree by the population mean number of healthy seed cones (Strauss & Irwin 2004; Salgado-Luarte & Gianoli 2012; Abdala-Roberts *et al.* 2014). Under both scenarios, relative fitness was calculated separately for each type of year, and selection analyses were based upon genotype means (Rauscher 1992). Although information is lost when using mean values across plants within each genotype, there are important advantages to performing genotypic (rather than individual-level) analyses. In particular, it is well established that using genotype means avoids environmentally induced correlations between measured and unmeasured traits that may influence estimates of plant fitness in phenotypic (*i.e.* individual-level) selection

analyses (Rausher 1992; Stinchcombe *et al.* 2002). Selection in the absence of pine–seed predator interactions (as computed above) can be viewed as a null model to compare against an alternative (real) scenario where seed predator effects on fitness are accounted for.

Second, we estimated linear genotypic selection on seed cone number by running simple linear regressions (Lande & Arnold 1983; Rausher 1992), separately for each year type and seed predation scenario, where relative fitness was predicted by cone number (Lande & Arnold 1983; Rausher 1992), for a total of four estimates of selection (one for each seed predation scenario by year type combination). Although positive selection on cone number is expected (*i.e.* this trait is a component of fitness), there is substantial inter-annual variation in cone output for the selected pine genotypes (see Results), which suggests the presence of trade-offs (*e.g.* between current and future reproduction) that could help to maintain genetic variation in reproductive output in this species. In addition, we expected that density-dependent seed predator attack would strengthen such selection, as individuals with higher reproductive output suffer from lower attack rates and this enhances fitness in the presence of seed predators. For each regression model, we calculated standardised selection gradients (β_{σ}) for each year type by multiplying original slope values by the population SD for cone number (Lande & Arnold 1983; Stinchcombe 2005; ter-Horst *et al.* 2015). We note that in these regressions, the target trait under selection (cone number) was also used to calculate relative fitness (cone number/population mean cone number), which results in a perfect correlation ($R^2 = 1$) between fitness and cone number under the null scenario of no seed predator effects. Nonetheless, we expect that despite this artefact the approach used was effective in testing for changes in selection once seed predator impacts are taken into account.

Finally, we tested for the independent and interactive effects of seed predators and masting on selection on seed cone number using ANCOVA (Strauss & Irwin 2004; Salgado-Luarte & Gianoli 2012; Abdala-Roberts *et al.* 2014). We ran a model testing for the effects of seed cone number, seed predation scenario (present *versus* absent), year type (mast *versus* non-mast), all two-way interactions, and the three-way interaction on relative fitness (calculated as above). Significant seed cone number \times year type and seed cone number \times seed predation scenario interactions indicate a change in strength of directional selection between year types or due to seed predator attack (respectively). Importantly, a three-way interaction indicates that the selective effect of seed predators on seed cone number is influenced by year type. If this three-way interaction was significant, we conducted follow-up ANCOVAs to test for a selective effect of seed predators separately within each year type. We predicted that directional selection by seed predators would be stronger during the mast year, because negative density dependence (*i.e.* satiation) in seed predator attack would be stronger for the mast year. As above, for these follow-up analyses we modelled relative fitness as dependent upon cone number, seed predation scenario and their interaction to test for changes in linear selection by seed predators between year types. We did not test for non-linear selection because selection on a fitness component would be expected to be linear and positive (rather than disruptive or stabilising) in order to maximise fitness. In all of the ANCOVA models, we included pine genotype as a random effect to account for using the same plants per genotype to

compute fitness values under each seed predation scenario and for each year type. In all cases, data were normally distributed. All ANCOVA models were performed with PROC MIXED, whereas simple regressions were performed in PROC REG (in both cases SAS version 9.4).

RESULTS

Patterns of seed cone production

Across all sampled trees, a total of 151,478 seed cones were recorded over the 6 years of study, with an average of $25,246 \pm 7,801$ cones produced per year (mean \pm SE). The site-wide total cone production varied extensively among the 6 years, ranging from 1,682 in one of the non-mast years to 56,259 seed cones in the mast year. Likewise, individual trees varied extensively in seed cone production, ranging from 0 to 648 total seed cones produced per tree across the 6-year period. There was significant genetic variation in total seed cone production over the 6 years of study ($F_{115,863} = 8.98$, $P < 0.0001$; Fig. 1a), with up to 325-fold variation in seed cone production among genotypes (genotype mean range: 1.9 ± 34.3 to 612.0 ± 32.6 cones; Fig. 1a).

Functional response of seed predators

A total of 18,902 seed cones were attacked by seed predators over the 6 years of study, with the annual mean proportion of attacked cones ranging from 0.09 to 0.17. In addition, we found genetic variation in the proportion of attacked seed cones over the sampling period ($F_{115,863} = 1.86$, $P < 0.0001$; Fig. 1b), with more than 30-fold variation among genotypes in the mean proportion of attacked seed cones (genotype mean range: 0.02 ± 0.08 to 0.63 ± 0.06 ; Fig. 1b).

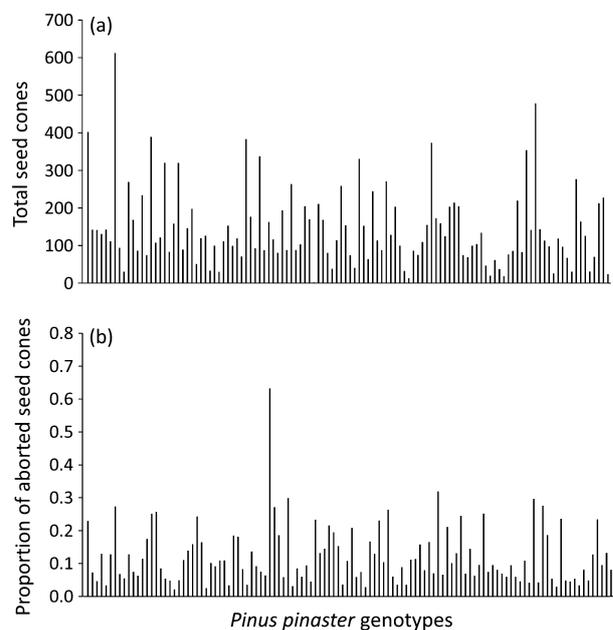


Fig. 1. *Pinus pinaster* genotype means ($N = 116$ genotypes) for (a) total seed cone production per tree, and (b) proportion of aborted seed cones per tree across the 6 years of study. Least-square means are shown ($N = 10$ individuals per genotype).

Table 1. Functional response of seed predators to availability of seed cones at the genotypic level (116 pine genotypes of *Pinus pinaster*) over the 6 years of study (a), and test of the change in seed predator attack rates (i.e. degree of negative density dependence) across year types (mast versus non-mast) (b). For 'b', a significant interaction between year type and seed cone number indicates that the slope of the relationship between proportion of attacked seed cones due to seed predators and seed cone number (i.e. functional response) is influenced by year type

effects	DF	parameter	SE	χ^2	<i>P</i>
a) across the 6 years of study					
cone number	1, 113	-0.0030	0.0006	24.46	<0.001
cone number ²	1, 113	0.0000	0.0000	38.21	<0.001
b) Mast versus non-mast years					
cone number	1, 225	-0.0064	0.0014	19.61	<0.001
cone number ²	1, 225	0.0000	0.0000	31.13	<0.001
year type	1, 225	0.4535	0.1394	10.58	0.001
year type × cone number	1, 225	0.0054	0.0026	4.47	0.034
year type × cone number ²	1, 225	-0.0000	0.0000	0.99	0.319

DF, degrees of freedom.

Logistic regressions were used to examine the linear and quadratic effects of cones on the proportion of attacked cones. Significant ($P < 0.05$) *P*-values are shown in bold.

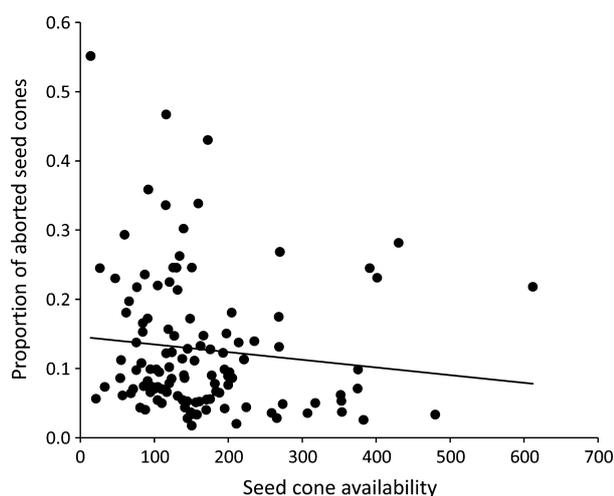


Fig. 2. Functional response of seed predators to seed cones produced by *Pinus pinaster*. The type of functional response is depicted by the relationship between the number of available seed cones and the predation rate (proportion of attacked seed cones) across the 6 years of study. Each point represents a genotype mean across years ($N = 116$ genotypes). Seed predators exhibited a Type II response (i.e. predator satiation), as indicated by a significant negative linear term for seed cone number (see Table 1a).

Our analysis indicated that seed predators exhibited a Type II response (i.e. predator satiation), as indicated by a significant negative linear term for the number seed cones (Table 1a, Fig. 2). In addition, we found that attack rates were weaker for the mast year (i.e. increased satiation) than for the mean across non-mast years. Specifically, we found a significant year type × seed cone interaction and the parameter for this term was positive (Table 1b), suggesting a lower proportion of attacked seed cones per seed cone produced during the mast year, i.e. seed predator satiation tended to be stronger during the mast year.

Table 2. Measurements of directional selection on *Pinus pinaster* seed cone number shown separately for each year type (i.e. mast versus non-mast years) and 'seed predation' scenario (i.e. seed predation effects on plant fitness present versus absent). Both standardised (β) and unstandardised (β_σ) selection gradients and SE are shown

effects	β (SE)	β_σ (SE)
mast year		
seed predation effects present	0.0172 (0.0004)*	0.63 (0.01)
seed predation effects absent	0.0123 (-)*	0.45 (-)
non-mast years		
seed predation effects present	0.0109 (0.0002)*	0.75 (0.01)
seed predation effects absent	0.0111 (-)*	0.77 (-)

Significant ($P < 0.05$) *P*-values are shown in bold.

* $P < 0.001$. SE could be estimated in the absence of seed predators as the target trait under selection (i.e. cone number) was also used to calculate relative fitness (cone number/population mean cone number), resulting in a perfect correlation ($R^2 = 1$) between fitness and cone number.

Effect of masting on seed predator selection

As expected, simple linear regressions indicated positive directional selection by seed predators on seed cone production, and this was true for both the mast and non-mast years as well as under both scenarios of seed predation (seed predator effects present versus absent; Table 2). The ANCOVA revealed significant seed predation scenario × cone number and year type × cone number interactions (Table 3), where the strength of positive directional selection on cone number was greater when seed predator effects were present and under non-mast years (Table 2). Furthermore, we found a significant seed predation scenario × year type × seed cone number interaction (Table 3). Subsequent exploratory ANCOVAs testing for seed predator selective effects separately within each year type indicated that directional selection on cone number during the non-masting year type was not influenced by the seed predation treatment (non-significant seed predation scenario × cone number interaction; $F_{1,227} = 1.29$, $P = 0.257$), i.e. seed predators did not exert a selective effect on cone output (similar linear selection coefficients; see Table 2, Fig. 3a). In contrast, for the mast year we found that the seed predation treatment influenced directional selection on cone number (significant seed predation scenario × seed cone interaction; $F_{1,227} = 150.32$, $P < 0.001$), with the strength of selection being higher in the presence than in the absence of seed predation effects (Table 2, Fig. 3b), i.e. seed predators strengthened selection on seed cone number.

DISCUSSION

Functional response of seed predators

Following the PSH, our analyses at the individual tree level indicated that the functional response of seed predators to *P. pinaster* seed cones was a Type II, negative density-dependent response (i.e. predator satiation), where the proportion of attacked cones decreased with increasing cone output. Type II functional responses are typical of relatively immobile specialist predators that feed on only one or a few prey species (Holling 1959, 1965). Accordingly, for 2003 (non-mast year) and 2004

(mast year) we recorded the number of attacked seed cones separately for each seed predator species (three specialist insects and two generalist vertebrates, see Methods) and found that, on average, *ca.* 85% of the attacked seed cones were attacked by specialist insects. Furthermore, we found that attack rates were lower in the mast year than during the non-mast years, indicating that masting enhances seed predator satiation. To date, only two previous studies have addressed the influence of mast seeding events on seed predator functional responses (Fletcher *et al.* 2010; Linhart *et al.* 2014). Fletcher *et al.* (2010) reported a Type III population-wide

functional response for generalist squirrels to seed cones of white spruce (*Picea glauca*), whereas Linhart *et al.* (2014) found a Type II population-wide functional response of specialist insects to available cones of ponderosa pine (*P. ponderosa*). In agreement with our results, these studies found that negative density-dependent attack by seed predators was either more pronounced (Fletcher *et al.* 2010) or only present (Linhart *et al.* 2014) during masting events.

Effect of masting on seed predator selection

Despite recent progress in our understanding of changes in patterns of seed predator attack due to masting events, the evolutionary consequences of such dynamics have been poorly studied. In this sense, our findings suggest that seed predators strengthened directional selection on the size of individual reproductive efforts of *P. pinaster* only during the mast year, a finding that is expected for seed predators exhibiting negative density-dependent attack because individual genotypes with higher reproductive output experience lower attack rates, and this enhances their relative fitness in the presence of seed predators (Leimu *et al.* 2002; Kolb & Ehrlén 2010; Abdala-Roberts & Mooney 2014). These results agree with Abdala-Roberts *et al.* (2014) who found that the proportion of fruits attacked by a seed predator in the perennial herb *Ruellia nudiflora* decreased with increasing fruit output, and as a result seed predators strengthened positive selection on fruit number (see also Leimu *et al.* 2002; Jones & Comita 2010). We must note, however, that an important limitation of our study is that we included only one mast year rather than multiple masting events, thus precluding a definitive test of the ecological and evolutionary effects of masting on pine–seed predator interactions. Despite this limitation, we still consider that our findings are suggestive of a potentially common, but understudied, form of context dependency in seed predator selective effects on plants *via* synchronised reproduction (*i.e.* masting behaviour). Accordingly, the patterns observed here can be

Table 3. Results from an ANCOVA model testing for changes in the strength of directional selection ($N = 116$ genotypes) on *Pinus pinaster* seed cone number under two ‘scenarios’ of seed predation effects (*i.e.* seed predation effects present versus absent) and two year types (mast versus non-mast years)

effects	DF	Parameter	SE	F-value	P
cone number	1, 456	0.0172	0.0002	15419.4	<0.001
seed predation	1, 456	−0.0217	0.0161	1.82	0.178
year type	1, 456	−0.0025	0.0162	0.02	0.876
seed predation × cone number	1, 456	−0.0049	0.0003	140.86	<0.001
year type × cone number	1, 456	−0.0063	0.0002	323.03	<0.001
seed predation × year type × cone number	1, 456	0.0051	0.0002	409.88	<0.001

DF, degrees of freedom.

Results for directional selection on cone number are from a model including seed predation treatment, year type, seed cone number, all two-way interactions and the three-way interaction. Pine genotype was considered as a random factor to account for using the same plants of each genotype to compute fitness values under each seed predation scenario and year type. Significant *P*-values are shown in bold.

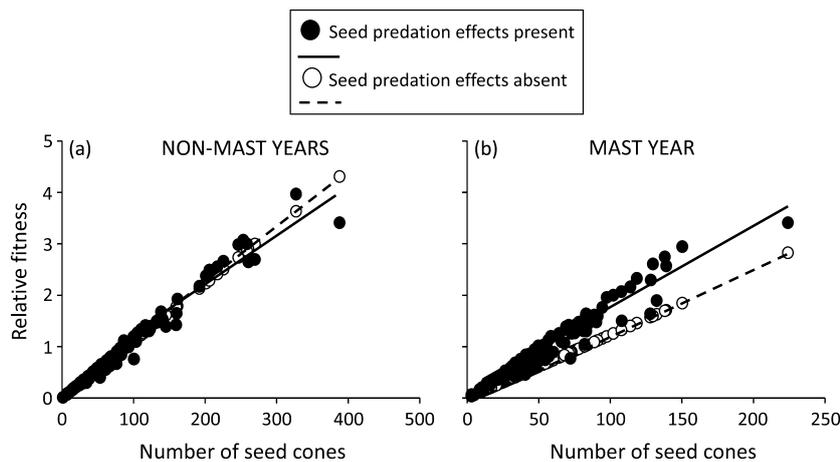


Fig. 3. Relationship between *Pinus pinaster* seed cone number and the relative fitness calculated under two ‘scenarios’ of seed predation effects (seed predation effects present in black dots and solid line versus seed predation effects absent in white dots and dashed line), shown separately for (a) non-mast years (using the sum across these years) and (b) one mast year. Seed predators select for increased seed cone output only during the mast year (significant seed predation scenario × seed cone interaction; $F_{1,227} = 150.32$, $P < 0.001$), which is when negative density dependence was more pronounced. Relative fitness is the number of seed cones of a given genotype divided by the population mean number of seed cones under each seed predation scenario. Each dot represents a single *P. pinaster* genotype ($N = 116$).

extended to other types of population dynamics involving synchronous responses to climatic cues (as for masting; Pearse *et al.* 2014; Moreira *et al.* 2015) and resource pulses (Chesson *et al.* 2004; Yang *et al.* 2008) that alter species interactions (Ostfeld & Keesing 2000; Polis *et al.* 2004; Friman & Laakso 2011). As such, these transient, synchronous responses across individuals in a population could influence the evolutionary outcomes of species interactions and provide opportunities for particularly strong episodes of selection, as shown in this study.

It is important to consider that the selective effects of seed predators on reproductive output in long-lived plants can only be fully addressed within the context of inter-annual reproductive allocation dynamics. Accordingly, testing the evolutionary mechanisms underpinning masting requires testing for selection on inter-annual variability. However, this requires exceptionally long-term datasets, whereas our study included only 6 years. Despite this, this study nonetheless tests for subsidiary predictions related to effects on variability. Masting is considered adaptive based on three inter-related phenomena: (i) large reproductive events lead to predator satiation (*i.e.* reduced attack rates); (ii) there are among-year trade-offs in reproductive investment, thus generating years of low output following years of high output (*i.e.* high inter-annual variation in reproductive output); and (iii) years of low investment starve (reduce population sizes) seed predators, thus increasing the efficacy of predator satiation during mast years. Following from this, we speculate that if trade-offs between current and future reproduction emerge in *P. pinaster*, then the selective effect of seed predators on cone output within years should, in turn, favour increased inter-annual variation in reproductive output (reinforcing, in a way, effects of trade-offs on variation), the hallmark of masting (Pearse *et al.* 2014; Moreira *et al.* 2015). Preliminary analyses using data from this study indicated that trade-offs between current and future reproduction were lacking in *P. pinaster* (X. Moreira *et al.*, unpublished data). However, for long-lived species such as pines such trade-offs may arise in the long term, and we would expect seed predator selection for increased cone output within a given year to influence patterns of reproductive investment in subsequent years (assuming reproductive trade-offs occur; Pearse *et al.* 2014; Moreira *et al.* 2015), and potentially lead to larger variation in reproductive output among years.

Although our results contribute to a growing literature supporting seed predator satiation as an important driver of masting behaviour (*e.g.* Fletcher *et al.* 2010; Archibald *et al.* 2012; Linhart *et al.* 2014; Mezquida *et al.* 2016), dynamics based on other types of economies of scale could also explain this reproductive phenomenon. For example, masting might increase pollination success through synchronised flowering effort (*i.e.* the 'Pollination Efficiency Hypothesis'; Kelly 1994; Moreira *et al.* 2014) or might improve seed dispersal through increased attraction of animal seed dispersers (*i.e.* the 'Animal Dispersal Hypothesis'; Kelly 1994; Zwolak *et al.* 2016). Accordingly,

much insight will be gained to the extent that future studies simultaneously assess the influence of multiple mechanisms potentially underlying masting in plants.

Concluding remarks and future directions

Our results suggest that synchronous reproduction leading to large seed crops in long-lived trees (*i.e.* masting) shapes plant–seed predator evolutionary dynamics. Masting led to lower rates of seed predator attack, and this in turn influenced natural selection by seed predators on seed cone production. These findings highlight that changes in consumer responses to resource abundance represent a potentially widespread mechanism for explaining and describing context dependency in plant–seed predator evolutionary dynamics. More generally, the dynamics outlined above need not to be restricted to masting *per se* and may span a variety of other episodic ecological dynamics characterised by synchronous population- or community-level responses. We therefore consider that a promising avenue of future research will be to investigate the evolutionary consequences of ecological dynamics driven by resource pulses and climatic cues, and how changes in species functional responses underlie such dynamics. For example, ecological transients such as plant and consumer responses to rainfall variability in deciduous forests and deserts, upwelling events in marine ecosystems, animal migrations or seasonal pulses of allochthonous resources in rivers, lakes and estuaries might drastically influence the strength of resource–consumer relationships and alter species interactions, and thus clearly deserve attention within an evolutionary context.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Information about the *Pinus pinaster* genetic trial. And also includes the figure S1 (types of functional responses) and the figure S2 (classification of mast and non-mast years).

REFERENCES

- Abdala-Roberts L., Mooney K.A. (2014) Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system. *Ecology*, **95**, 2879–2893.
- Abdala-Roberts L., Mooney K.A. (2015) Plant and herbivore evolution within the trophic sandwich. In: Hanley T. C. N., La Pierre K. J. (Eds), *Trophic interactions: bottom-up and top-down interactions in aquatic and terrestrial ecosystems*. Cambridge University Press, Cambridge, UK, pp 339–363.
- Abdala-Roberts L., Parra-Tabla V., Campbell D.R., Mooney K.A. (2014) Soil fertility and parasitoids shape herbivore selection on plants. *Journal of Ecology*, **102**, 1120–1128.
- Abrahamson W.G. (1989) *Plant–animal interactions*. McGraw-Hill, New York, NY, USA.

- Abrams P.A. (1982) Functional responses of optimal foragers. *The American Naturalist*, **120**, 382–390.
- Abrams P.A. (2000) The evolution of predator–prey interactions: theory and evidence. *Annual Review of Ecology, Evolution and Systematics*, **31**, 79–105.
- Agrawal A.A. (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*, **7**, 651–667.
- Archibald D.W., McAdam A.G., Boutin S., Fletcher Q.E., Humphries M.M. (2012) Within-season synchrony of a masting conifer enhances seed escape. *The American Naturalist*, **179**, 536–544.
- Bogdziewicz M., Zwolak R., Crone E.E. (2016) How do vertebrates respond to mast seeding? *Oikos*, **125**, 300–307.
- Chesson P., Gebauer R.L.E., Schwinning S., Huntly N., Wiegand K., Ernest M.S.K., Sher A., Novoplansky A., Weltzin J.F. (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia*, **141**, 236–253.
- Espelta J.M., Bonal R., Sánchez-Humanes B. (2009) Pre-dispersal acorn predation in mixed oak forests: Interspecific differences are driven by the interplay among seed phenology, seed size and predator size. *Journal of Ecology*, **97**, 1416–1423.
- Fletcher Q.E., Boutin S., Lane J.E., LaMontagne J.M., McAdam A.G., Krebs C.J., Humphries M.M. (2010) The functional response of a hoarding seed predator to mast seeding. *Ecology*, **91**, 2673–2683.
- Friman V.-P., Laakso J. (2011) Resource dynamics constrain the evolution of predator–prey interactions. *The American Naturalist*, **177**, 334–345.
- Gómez J.M. (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *The American Naturalist*, **162**, 242–256.
- Herrera C.M., Medrano M., Rey P.J., Sanchez-Lafuente A.M., Garcia M.B., Guitián J., Manzaneda A.J. (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences USA*, **99**, 16823–16828.
- Holling C.S. (1959) The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Canadian Entomologist*, **91**, 293–320.
- Holling C.S. (1965) The functional response of predators to prey density and its role in mimicry and population regulation. *Memoirs of the Entomological Society of Canada*, **48**, 3–86.
- terHorst C.P., Lau J.A., Cooper I.A., Keller K.R., La Rosa R.J., Royer A.M., Schultheis E.H., Suwa T., Conner J.K. (2015) Quantifying non-additive selection caused by indirect ecological effects. *Ecology*, **96**, 2360–2369.
- Janzen D.H. (1976) Why bamboos wait so long to flower. *Annual Review of Ecology and Systematics*, **7**, 347–391.
- Johnson M.T.J., Stinchcombe J.R. (2007) An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology & Evolution*, **22**, 250–257.
- Jones F.A., Comita L.S. (2010) Density-dependent pre-dispersal seed predation and fruit set in a tropical tree. *Oikos*, **119**, 1841–1847.
- Juliano S.A. (2001) Non-linear curve fitting: predation and functional response curves. In: Scheiner S. M., Gurevitch J. (Eds.), *Design and analysis of ecological experiments*. Oxford University Press, New York, USA, pp 178–196.
- Kelly D. (1994) The evolutionary ecology of mast seeding. *Trends in Ecology & Evolution*, **9**, 465–470.
- Kolb A., Ehrlén J. (2010) Environmental context drives seed predator-mediated selection on a floral display trait. *Evolutionary Ecology*, **24**, 433–445.
- LaMontagne J.M., Boutin S. (2009) Quantitative methods for defining mast-seeding years across species and studies. *Journal of Vegetation Science*, **20**, 745–753.
- Lande R., Arnold R. (1983) The measurement of selection on correlated characters. *Evolution*, **37**, 1210–1226.
- Leimu R., Syrjänen K., Ehrlén J., Lehtilä K. (2002) Pre-dispersal seed predation in *Primula veris*: among-population variation in damage intensity and selection on flower number. *Oecologia*, **133**, 510–516.
- Linhart Y.B., Moreira X., Snyder M.A., Mooney K.A. (2014) Variability in seed cone production and functional response of seed predators to seed cone availability: support for the predator satiation hypothesis. *Journal of Ecology*, **102**, 576–583.
- Littell R.C., Milliken G.A., Stroup W.W., Wolfinger R., Schabenberger O. (2006) *SAS System for mixed models* 2nd edn. SAS, Cary, NC, USA.
- Maron J.L., Baer K.C., Angert A.L. (2014) Disentangling the drivers of context-dependent plant–animal interactions. *Journal of Ecology*, **102**, 1485–1496.
- Mezquida E.T., Rodríguez-García E., Olano J.M. (2016) Efficiency of pollination and satiation of predators determine reproductive output in Iberian *Juniperus thurifera* woodlands. *Plant Biology*, **18**, 147–155.
- Miller T.E.X., Tenhumberg B., Louda S.M. (2008) Herbivore-mediated ecological costs of reproduction shape the life history of an iteroparous plant. *The American Naturalist*, **171**, 141–149.
- Moreira X., Abdala-Roberts L., Linhart Y.B., Mooney K.A. (2014) Masting promotes individual- and population-level reproduction by increasing pollination efficiency. *Ecology*, **95**, 801–807.
- Moreira X., Abdala-Roberts L., Linhart Y.B., Mooney K.A. (2015) Effects of climate on reproductive investment in a masting species: assessment of climatic predictors and underlying mechanisms. *Journal of Ecology*, **103**, 1317–1324.
- Ostfeld R.S., Keesing F. (2000) Pulsed resources and community dynamics of consumers in terrestrial ecosystems. *Trends in Ecology & Evolution*, **15**, 232–237.
- Pearse I.S., Koenig W.D., Knops J.M.H. (2014) Cues versus proximate drivers: testing the mechanism behind masting behavior. *Oikos*, **123**, 179–184.
- Pesendorfer M.B., Koenig W.D. (2016) The effect of within-year variation in acorn crop size on seed predation and dispersal by avian hoarders. *Oecologia*, **181**, 97–106.
- Polis G.A., Power M.E., Huxel G.R. (2004) *Food webs at the landscape level*. University of Chicago Press, Chicago, IL, USA.
- Price P.W., Lewinsohn T.M., Fernandes W., Benson W.W. (1991) *Plant–animal interactions: evolutionary ecology in tropical and temperate regions*. John Wiley and Sons, New York, NY, USA.
- Rausher M.D. (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*, **46**, 616–626.
- Rausher M.D., Simms E.L. (1989) The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. *Evolution*, **43**, 563–572.
- Richardson D.M. (1998) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge, UK, pp.556.
- Salgado-Luarte C., Gianoli E. (2012) Herbivores modify selection on plant functional traits in a temperate rainforest understory. *The American Naturalist*, **180**, E42–E53.
- Siepielski A.M., Benkman C.W. (2008) Seed predation and selection exerted by a seed predator influence subalpine tree densities. *Ecology*, **89**, 2960–2966.
- Silvertown J.W. (1980) The evolutionary ecology of mast-seeding in trees. *Biological Journal of the Linnean Society*, **14**, 235–250.
- Sletvold N., Moritz K.K., Ågren J. (2015) Additive effects of pollinators and herbivores result in both conflicting and reinforcing selection on floral traits. *Ecology*, **96**, 214–221.
- Stinchcombe J.R. (2005) Measuring natural selection on proportional traits: comparisons of three types of selection estimates for resistance and susceptibility to herbivore damage. *Evolutionary Ecology*, **19**, 363–373.
- Stinchcombe J.R., Rausher M.D. (2002) The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. *Proceedings of the Royal Society, Series B*, **269**, 1241–1246.
- Stinchcombe J.R., Rutter M.T., Burdick D.S., Tiffin P., Rausher M.D., Mauricio R. (2002) Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *The American Naturalist*, **160**, 511–523.
- Strauss S.Y., Irwin R.E. (2004) Ecological evolutionary consequences of multi-species interactions. *Annual Review of Ecology, Evolution, & Systematics*, **35**, 435–466.
- Thompson J.N. (1999) The evolution of species interactions. *Science*, **284**, 2116–2118.
- Trexler J.C., McCulloch C.E., Travis J. (1988) How can the functional response best be determined? *Oecologia*, **76**, 206–214.
- Valverde P.L., Fornoni J., Núñez-Farfán J. (2001) Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. *Journal of Evolutionary Biology*, **14**, 424–432.
- Yang L.H., Bastow J.L., Spence K.O., Wright A.N. (2008) What can we learn from resource pulses? *Ecology*, **89**, 621–634.
- Zwolak R., Bogdziewicz M., Wróbel A., Crone E.E. (2016) Advantages of masting in European beech: timing of granivore satiation and benefits of seed caching support the predator dispersal hypothesis. *Oecologia*, **180**, 749–758.