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Regulation of propagule size in the aquatic pseudo-annual *Potamogeton pectinatus*: are genetic and maternal non-genetic effects additive?

Helen H. Hangelbroek[1,2]* and Luis Santamaría[1]‡

[1]*Department of Plant–Animal Interactions, Netherlands Institute of Ecology (NIOO-KNAW), Rijksstraatweg 6, 3631 AC Nieuwersluis and* [2]*Department of Ecology, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*

ABSTRACT

Genetic and maternal non-genetic effects interact in shaping the phenotype of a particular trait. The strength of the genetic component determines whether selection pressure results in evolutionary changes in the population. The strength of the maternal non-genetic component can affect the pace of selection. In this study, we analysed genetic and maternal propagule size effects on propagule size production in the pondweed *Potamogeton pectinatus*. In particular, we analysed whether they interact significantly (i.e. whether both effects are additive, synergistic or antagonistic) and how they may influence the outcome of diversifying selection pressures in the field. Fifteen clones differing in the genetically determined size of asexual propagules (tubers) were grown for three asexual generations in a common-garden set-up. The first generation was grown from tubers collected from the field, the second from maternal tubers of comparable size, and the last from both small and large maternal tubers. Maternal tuber size had a large effect on all clones that was independent of their genetically determined tuber size – that is, genetic and maternal non-genetic effects were additive. Path analysis revealed that maternal tuber size affected tuber size and number similarly through its effect on biomass production (vegetative and total tuber production), while the genetic component had a direct effect on tuber size, associated with a trade-off with tuber number. Because the relationship between genetic and maternal non-genetic effects is additive, the outcome of diversifying selection related to tuber predation pressure by Bewick's swans and sediment heterogeneity will not be affected. However, since the maternal effect is large, variation around optimal sizes is likely to persist in the population, which is consistent with what is found in the field.

Keywords: carry-over effects, fennel pondweed, propagule size, size–number trade-off, tuber size.

* Address all correspondence to Helen H. Hangelbroek, Department of Ecology, Evolution and Behavior, University of Minnesota, 1987 Upper Bufurd Circle, 100 Ecology Building, St. Paul, MN 55108, USA. e-mail: hange001@umn.edu

‡ *Present address*: Mediterranean Institute for Advanced Studies, (IMEDEA, CSIC-UIB), c/ Miguel Marquès 21, 07190 Esporles, Mallorca, Balearic Islands, Spain.

INTRODUCTION

Propagule size varies considerably among plant species, with recorded sizes ranging from 2×10^{-6} g in the orchid *Goodyera repens* (Salisbury, 1942) to 18,000–27,000 g in the double coconut palm *Lodoicea maldivica* (Corner, 1966). It is not surprising that different plant species, with often distinct ecological strategies, show a large assortment of propagule sizes. Within species, on the other hand, propagule size is considered to be relatively stable (Harper *et al.*, 1970; Lloyd, 1987), with variation within one order of magnitude (Schaal, 1980; Banovetz and Scheiner, 1994; Eriksson, 1999; Susko and Lovett-Doust, 2000). Within species, selection may favour different sizes of propagules depending on local ecological conditions. Contrasting selection pressures on propagule size generally result from differences in propagule and seedling survival, growth, dispersal, predator avoidance and competition (Black, 1958; Schaal, 1980; Wulff, 1986; Van Groenendael and Habekotté, 1988; Ganeshaiah and Uma Shaanker, 1991; Moegenburg, 1996; Vera, 1997; Vaughton and Ramsey, 1998; Eriksson, 1999; Santamaría and Rodríguez-Gironés, 2002). But as for any other trait, natural selection not only requires phenotypic variation resulting in differential fitness effects, but also that such variation has a heritable component. Nonetheless, most studies on propagule size have focused on its potential benefits or disadvantages in terms of propagule survival and future plant performance, rather than on its heritability. The studies that have analysed the genetic basis of propagule size have reported varying results. Some have indicated strongly that propagule size has a genetic component and maternal environment has little effect on propagule size (Weiner *et al.*, 1997); others have shown that heritability is low and that maternal non-genetic effects play an important role (Schaal, 1980; Montalvo and Shaw, 1994); and yet others have shown that both genetic as well as maternal non-genetic effects contribute to propagule size variation (Schmitt *et al.*, 1992; Platenkamp and Shaw, 1993). Furthermore, several studies have shown low additive genetic variance (based on nuclear genes) for propagule size and high maternal variance (Mazer, 1987; Wolfe, 1995), which may either be the result of maternal non-genetic or maternal genetic effects. In the latter case, evolution of seed size may also take place (Platenkamp and Shaw, 1993; Montalvo and Shaw, 1994).

When both maternal non-genetic effects and genetic effects determine the phenotype of a certain trait, maternal non-genetic effects will increase the variation of phenotypes of each given genotype and thus could decrease the pace of natural selection (Roach and Wulff, 1987). The decrease in response to selection, however, need not be similar over the whole range of genotypes. Instead, it is likely to depend on the type of interaction that exists between genetic and maternal non-genetic effects. A maternal non-genetic effect may be comparable across genotypes (i.e. genetic and maternal non-genetic effects are additive; Fig.1b) or the maternal non-genetic effect may differ depending on the genotype (significant genotype × maternal environment interaction). In the second case, the maternal non-genetic effect may either amplify the value of the genetic trait (synergistic interaction; Fig. 1c) or it may counteract it (antagonistic interaction; Fig. 1d), resulting in differential strengths of response to selection pressure across genotypes. Gaining insight into the type of relationship between genetic and maternal non-genetic effects is thus essential in understanding and predicting potential responses to selection.

In this study, we analysed the contribution of genetic and maternal non-genetic effects and their interaction to the regulation of propagule size of the pondweed *Potamogeton pectinatus*, and how this influences the outcome of diversifying selection on propagule

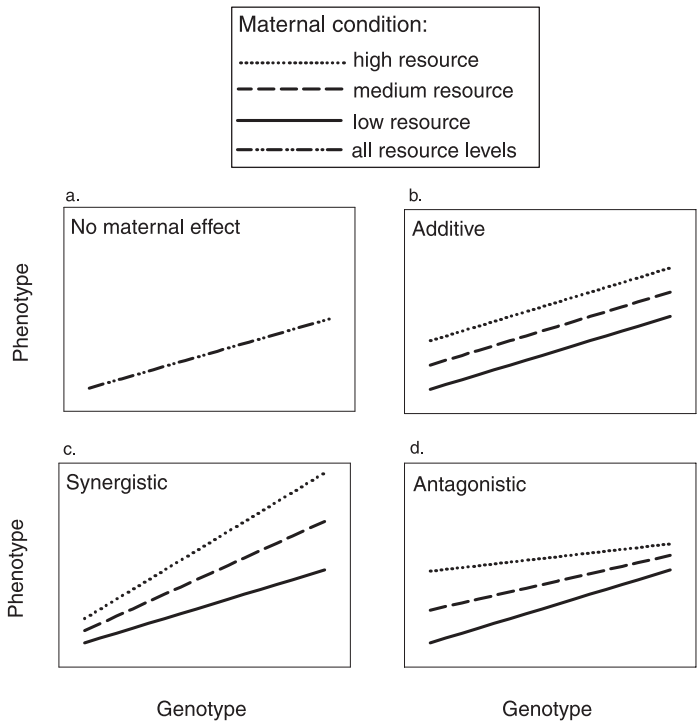

Fig. 1. Schematic representation of phenotype dependence on the type of interaction between genotype and maternal non-genetic conditions. (a) No maternal effects. (b) Additive relationship. (c) Synergistic interaction. (d) Antagonistic interaction. Maternal conditions may, for instance, represent differences in nutrient concentration of the maternal environment or differences in the propagule size from which a mother plant grows (maternal propagule size).

size. *Potamogeton pectinatus* is an aquatic macrophyte, which reproduces primarily by means of subterranean asexual propagules (tubers; Van Wijk, 1989). The size of these produced tubers has a large genetic component ($H^2 = 1.01$ broad sense heritability; H.H. Hangelbroek, L. Santamaría and M.A. Rodríguez-Gironés, unpublished manuscript). Maternal tuber size also influences produced tuber size (Rodríguez-Gironés *et al.*, 2003), but whether this effect varies across genotypes (i.e. interacts with the genetic component) is unknown. The tubers of *P. pectinatus* are subjected to several selective forces, such as size-selective predation by Bewick's swans and size-selective sprouting mortality, which are both related to burial depth in the sediment (larger tubers have larger burial depths; Santamaría and Rodríguez-Gironés, 2002). When spatial variation in sediment type occurs, different strategies for optimal tuber size may exist, since sediment type (clay content) is known to influence both predation pressure (Nolet *et al.*, 2001) and sprouting mortality (Santamaría and Rodríguez-Gironés, 2002). In sandy sediment where predation pressure and sprouting mortality are high, deeply buried large tubers are likely to be selected because deeply buried tubers may escape predation and large tuber sizes show reduced sprouting mortality. In clay-rich sediment, on the other hand, reduced predation pressure and tuber mortality accompanied by a size–number trade-off will most likely favour the production of many small tubers (Santamaría and Rodríguez-Gironés, 2002). However, maternal non-genetic

effects may affect the phenotype of the genetically determined tuber size, slowing down selection or, in the case of a significant maternal × genotypic interaction, affecting the outcome of selection. If, for instance, the interaction were antagonistic (i.e. tubers from clones determined to produce small tubers being more strongly affected by maternal tuber size than those being produced from clones with a larger genetic determined tuber size), then natural selection of large genotypes would occur more rapidly and small genotypes may eventually disappear from the population.

Here we studied the effect of genotypic variation and experimentally manipulated maternal non-genetic tuber size on produced tuber size. The main questions asked were: Does maternal tuber size affect the size of produced tubers? If so, what kind of interaction exists between the genetic component determining tuber size and the maternal effect: no interaction (additive effects), synergistic interaction or antagonistic interaction? What might the consequences of this particular interaction be on the diversifying selection pressures observed in the field? Furthermore, to gain better insight into the mechanistic processes regulating tuber size, we sought an answer to the following questions: Do genotypic variation and maternal tuber size affect other plant traits (such as tuber production or shoot-to-root ratio), which may in turn affect tuber size? Through which pathways (i.e. traits) do maternal tuber size and genetic effects regulate tuber size?

MATERIALS AND METHODS

Species and study system

Potamogeton pectinatus L. (fennel pondweed) is a submerged aquatic angiosperm that has a cosmopolitan distribution ranging from the sub-arctic to the tropics (Casper and Krausch, 1980; Wiegleb and Kaplan, 1998). It has a pseudo-annual life form: every year at the end of the growth season, the plants die off leaving below-ground asexual propagules (tubers) separated from one another to survive the winter and sprout in spring. *Potamogeton pectinatus* also reproduces sexually, but seeds are thought mainly to contribute to seed-bank build-up, population re-establishment after disturbances and waterfowl-mediated dispersal among water bodies (Van Wijk, 1989; Santamaría, 2002).

Plant material for this study was collected in the Babbelaar (Lake Lauwersmeer, the Netherlands), where a population of *P. pectinatus* occupies a heterogeneous area in terms of sediment type, water depth and foraging pressure by Bewick's swans (Nolet *et al.*, 2001). Previous studies have revealed that these factors affect different determinants of fitness in this species, including tuber production, winter survival of tubers and sprouting survival (Nolet *et al.*, 2001; Santamaría and Rodríguez-Gironés, 2002; Hangelbroek *et al.*, 2003).

Plant cultivation

We used 15 clonal lines for the experiment selected from an initial sample of 90 clones collected from the Babbelaar, using a criterion that maximized genotypic tuber size variation while maintaining the original number of five sampling sites (Santamaría and Rodríguez-Gironés, 2002): following one generation of growth under common-garden conditions, we selected clones producing the largest, medium and smallest average-tuber-size within each sampling site (medium tuber size was the closest to the grand-average over the five sampling sites). Clonal lines were then grown for a second asexual generation under

standardized common-garden conditions to minimize the influence of carry-over effects of the maternal environment (*sensu* Rossiter, 1996) other than those mediated by tuber size on the experiment reported here. The second generation was grown from maternal tubers of comparable size, so that differences in produced tuber size were mainly genetically based. Average tuber size produced by each clonal line in this second clonal generation ($n = 8$ plants per clone) was used as a measure of genetically determined tuber size and will be referred to hereafter as 'genotypic-tuber-size' (Fig. 2). Amplified fragment length polymorphism (AFLP) analysis was performed according to Vos *et al.* (1995), to test whether all clonal lines indeed represented different genets. All clones were distinguished from one another using the primer combination: *Eco*RI + ACC / *Mse*I + CTT.

Ninety tubers originally collected at five sampling sites in the field in April 1997 were stored in a refrigerator (at 4°C and in the dark) to continue hibernation until May 1997, when they were weighed individually (fresh weight, accuracy 1 mg) and planted in 5.5-litre pots containing a mixture of river sand and commercial potting clay (3 : 1 dry weight ratio). Pots were randomly interspersed in five outdoor tanks (18 pots per tank) filled with tap water in Heteren (the Netherlands). The plants were left to grow until the end of the growing season (October 1997), when newly produced tubers were harvested. These tubers were weighed individually and stored until May 1998 (as above).

In the second year, eight tubers of each clone were planted separately in 5.5-litre pots with a sediment mixture of aquarium sand and commercial potting clay (2 : 1 dry weight ratio) (Fig. 2). The size of these eight tubers was standardized to a comparable size range for all clones (15–90 mg fresh weight ≈5–30 mg dry weight). Pots were randomly interspersed with pots from another experiment containing resource-poor sediment and divided among 12 outdoor tanks (20 pots per tank). They were left to grow until October 1998, when newly produced tubers were harvested, individually weighed and stored (as above). Throughout the experiment, tuber dry weights were calculated using linear regressions of dry weight (dw) on fresh weight (fw), estimated from a randomly chosen sub-sample of tubers from all clonal lines (year 1: $\mathrm{dw} = 0.34 \times \mathrm{fw}$, $R^2 = 0.95$, $n = 60$; year 2: $\mathrm{dw} = 0.35 \times \mathrm{fw}$, $R^2 = 0.95$, $n = 210$; dry weight was measured after 24 h of desiccation at 70°C). The genotypic-tuber-size for each clonal line was calculated as the average across all separate plants (ramets) of the geometric mean size of all tubers produced by each plant (log transformation before averaging was necessary because the distribution of tuber sizes produced by one plant is right-skewed).

Experimental design

To analyse the relationship between genetic and maternal non-genetic effects of tuber size on newly produced tuber size and other plant traits, a third year of growth in common-garden conditions was carried out with the 15 clones differing in genotypic-tuber-size, only now plants from all clonal lines were grown from tubers belonging to two different, non-overlapping size categories ('maternal-tuber-size' classes) (Fig. 2). From each clone, we selected 10 small (11–20 mg fw = 4–7 mg dw) and 10 large (80–126 mg fw = 28–44 mg dw) tubers. In May 1999, these tubers were planted in 5.5-litre pots containing a sediment mixture of aquarium sand and commercial potting clay (3 : 1 dry weight ratio) and randomly interspersed among 15 outdoor tanks (20 pots per tank). The experimental set-up resulted in a total of 300 pots (15 clones × 2 maternal-tuber-size classes × 10 replicates). In October 1999, plants were harvested and divided into above-ground (shoots + leaves) and

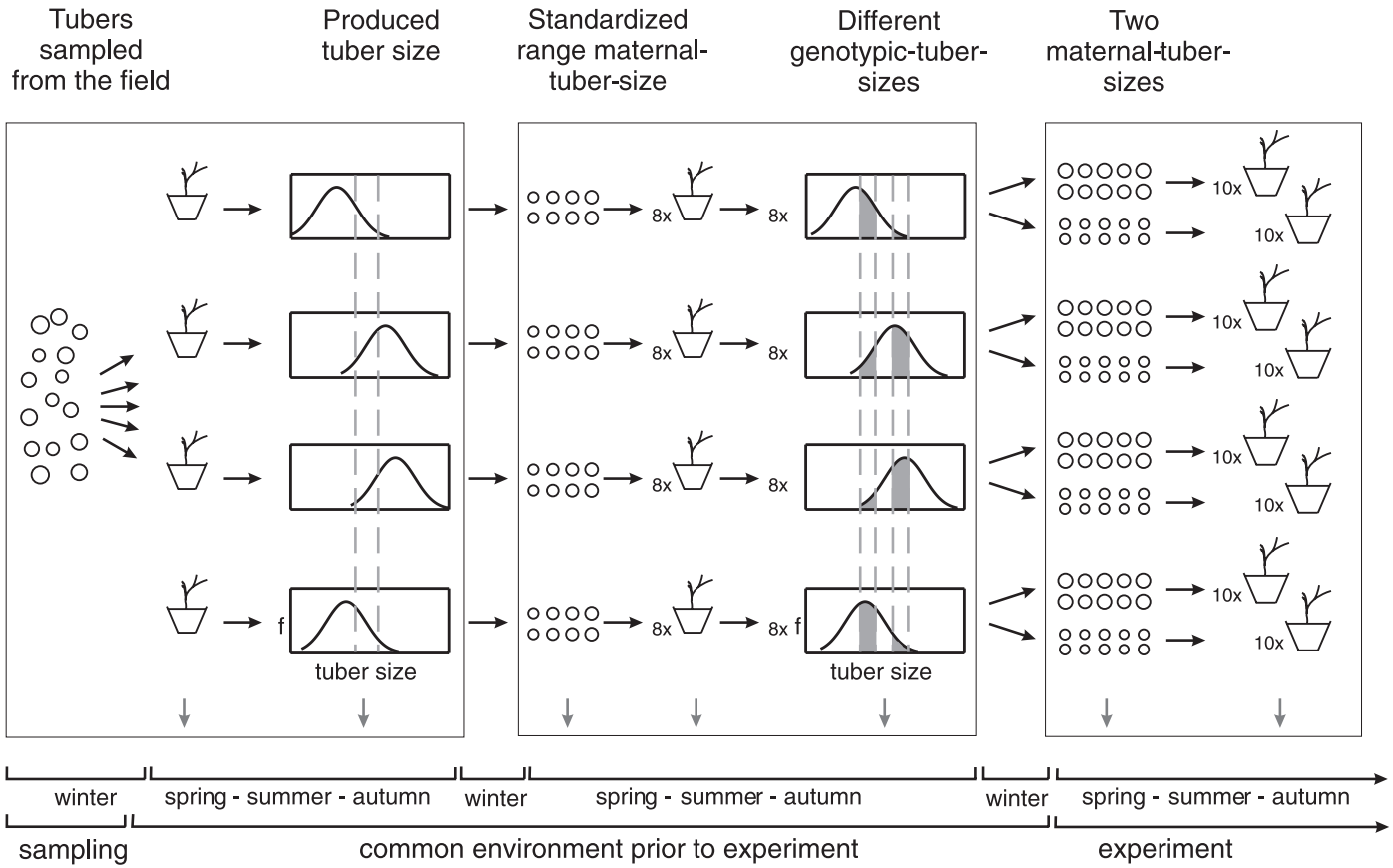

Fig. 2. Experimental design. Fifteen clonal lines obtained from 15 tubers collected from the field were grown for three asexual generations in common-garden conditions. In the first year, maternal-tuber-size differed among clones. In the second year, maternal-tuber-size was standardized for all clones. Clonal variation in (average) produced tuber size thus represents genetic differences among clones. In the third year, the 15 clones were grown from either small or large maternal tubers. Dashed lines represent the range of tuber sizes used as maternal tubers in the next growing season. For simplicity, only four of the 15 clones are depicted.

below-ground (roots + rhizomes) vegetative fractions, and tubers for biomass determination (fresh weight for the tubers, dry weight for the rest). Individual tuber fresh weights were measured and recalculated to individual dry weights using fresh- to dry-weight regressions estimated from a sub-sample of tubers (as above; dw = 0.39 × fw, $R^2 = 0.96$, $n = 75$). We then calculated total tuber production (sum of all individual tuber weights), vegetative biomass (above-ground + below-ground fractions), shoot-to-root ratio (above-ground biomass/ below-ground biomass) and allocation to asexual reproduction (tuber production/ vegetative biomass + tuber production).

Data analysis

Genotypic and maternal non-genetic effects on asexual reproductive traits (total tuber production, tuber size and number), biomass yield and allocation were tested in three steps. In all analyses, all variables were log $(x + 1)$ transformed (except for tuber number, which was square root transformed) to ensure homoscedasticity and normality of residuals.

First, we assessed whether the effect of maternal tuber size varied among clones by means of mixed-model analyses of variance, with maternal-tuber-size as a fixed categorical factor and clonal identity as a random categorical factor. A significant interaction between maternal-tuber-size and clone was interpreted to reveal genotypic variation in maternal effects (similar to the analysis of reaction norms in a plasticity experiment). To account for the influence of environmental variation between tanks, 'tank' was included as a random categorical factor.

Second, we evaluated whether among-clone variation in the effect of maternal-tuber-size was related to their genetically based differences in tuber size, by means of mixed-model analyses of covariance, with genotypic-tuber-size as a continuous factor, maternal-tuber-size as a fixed categorical factor and tank as a random categorical factor. First, analyses of covariance including the interaction between maternal and genotypic-tuber-size revealed that this term was always non-significant; hence, we carried out a second set of analyses of covariance without the interaction term. All analyses of variance and analyses of covariance were performed using the General Linear Models module of Statistica 6.0 (StatSoft, 2001).

Third, we sought to reveal the direct and indirect paths through which maternal-tuber-size and genotypic-tuber-size influence newly produced tuber size, by means of path analyses based on partial correlation coefficients obtained from a hierarchical set of multiple regressions (Huber *et al.*, 1996; Hangelbroek *et al.*, 2002; Santamaría and Rodríguez-Gironés, 2002).

RESULTS

Maternal-tuber-size, clonal origin and their interaction had significant effects on all traits, except for the effect of maternal-tuber-size on asexual reproductive allocation (Table 1). Whenever maternal-tuber-size resulted in significant effects, increased maternal-tuber-size led to an increase in the measured variable, except for shoot-to-root ratio, which decreased (Fig. 3). The significant interaction term indicated that, although the direction of the responses to maternal-tuber-size was generally similar across all clones, the magnitude of the responses differed (Fig. 3).

Table 1. *F*-ratios and significance of mixed-model analyses of variance fitted by generalized linear modelling

	MTS (d.f. = 1,14)	Clone (d.f. = 14,14)	MTS × clone (d.f. = 14,232–237)	Tank (d.f. = 14,232–237)
Asexual reproduction				
Tuber production[a]	40.84***	2.78*	2.72***	1.95*
Tuber number[b]	27.07***	3.92**	1.90*	3.18***
Average tuber size[c]	32.07***	7.57***	2.72***	1.91*
Biomass yield and allocation				
Vegetative biomass[a]	18.15***	2.71*	5.38***	1.92*
Asexual reproductive allocation[d]	0.03	3.02*	3.37***	3.50***
Shoot-to-root ratio[a]	35.70***	2.58*	2.01*	5.98***

Note: Maternal-tuber-size (MTS) represents two size categories of tubers from which plants were grown from. Clone is a random factor representing the clonal line the plants belong to. Tank is a random factor. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[a] Log($x + 1$). [b] Square root. [c] Individual tuber sizes log($x + 1$) transformed before averaging per plant. [d] Arcsin square root.

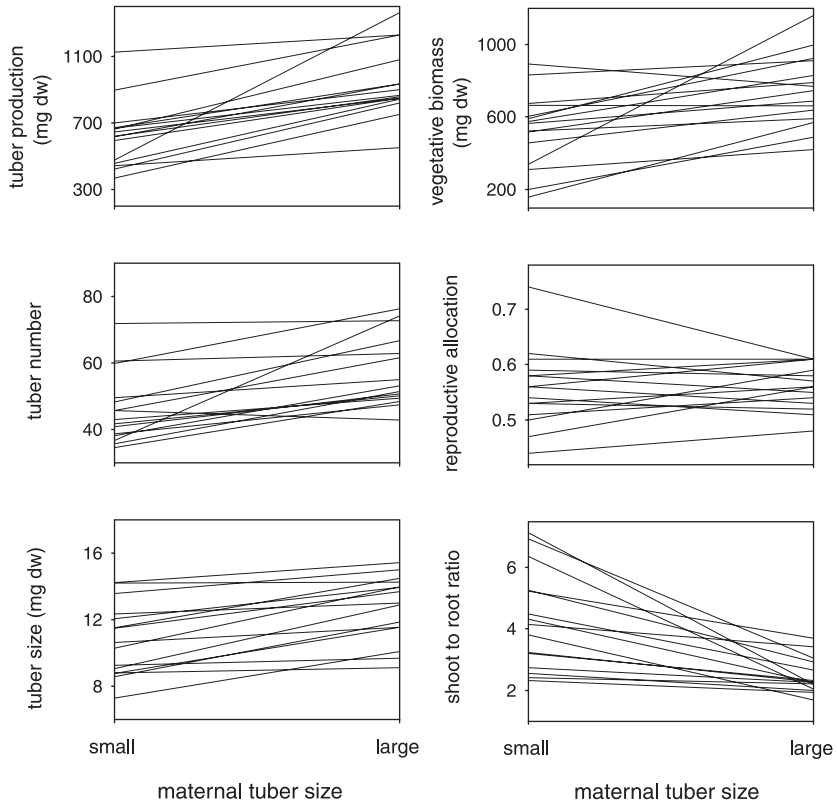

Fig. 3. Reaction norms of 15 *Potamogeton pectinatus* clones grown from small (5.7 ± 0.1 mg dw; mean ± standard error) and from large (34.1 ± 0.3 mg dw) maternal tubers (n = 5–10).

The first set of analyses of covariance including the interaction between maternal and genotypic-tuber-size revealed that this term was always non-significant ($P > 0.05$), indicating homogeneity of slopes between maternal-tuber-sizes (Fig. 4bc). The second set of analyses of covariance revealed that both maternal-tuber-size and genotypic-tuber-size had significant effects on produced tuber size and tuber number (Table 2, Fig. 4b,c). Total tuber production, however, was only affected by maternal-tuber-size (Table 2), being larger for plants grown from large maternal tubers (Fig. 4a). Not surprisingly, plants grown from large tubers produced more and larger tubers than plants grown from smaller tubers (Fig. 4b,c).

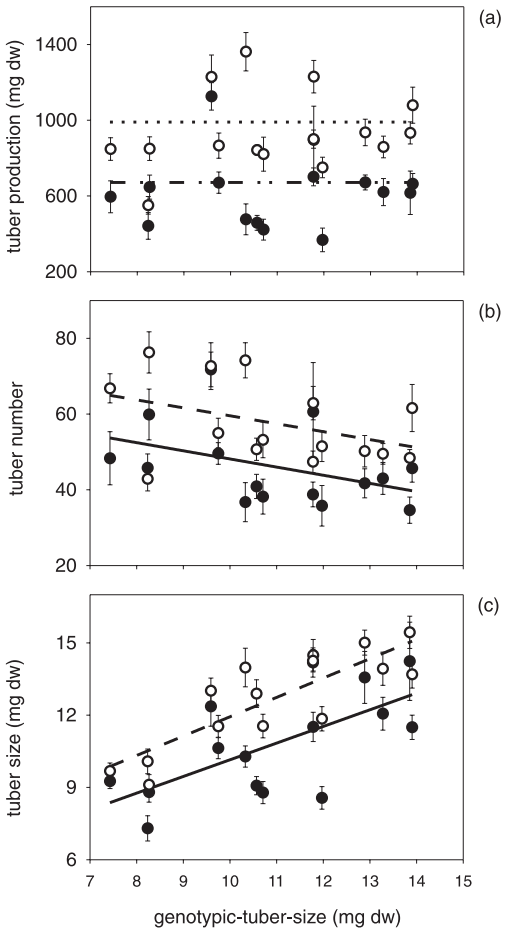

Fig. 4. Variation in asexual reproductive traits of 15 *Potamogeton pectinatus* clones that show genetic variation in tuber size. Plants were grown from either small (●) or large (○) maternal tubers. (a) Tuber production per plant (mg dw). (b) Number of tubers per plant. (c) Average tuber size per plant (mg dw). In (b) and (c), the lines depict the relationship between the variables (averaged per clone) and the genotypic-tuber-size of the various clones. Solid lines represent plants grown from small maternal tubers; dashed lines represent plants grown from large maternal tubers. Because tuber production is not affected by genotypic-tuber-size, the two lines in (a) represent the average tuber production of all clones grown from small (dashed-dotted) or large (dotted) maternal tubers.

Table 2. *F*-ratios and significance of analyses of covariance fitted by generalized linear modelling

	MTS (d.f. = 1,259–264)	GTS (d.f. = 1,259–264)	Tank (d.f. = 14,259–264)
Asexual reproduction			
Tuber production[a]	73.57***	1.84	1.01
Tuber number[b]	34.93***	14.11***	2.19**
Average tuber size[c]	58.02***	112.06***	1.73*
Biomass yield and allocation			
Vegetative biomass[a]	45.30***	0.33	1.71
Asexual reproductive allocation[d]	0.31	6.83**	4.72*
Shoot-to-root ratio[a]	47.49***	2.42	5.68***

Note: Maternal-tuber-size (MTS) is a fixed factor that represents two size categories of tubers from which plants were grown. Genotypic-tuber-size (GTS; i.e. genetically determined tuber size) is a continuous covariate, which is based on the average size of produced tubers after two clonal generations under standardized conditions and grown from tubers of comparable size. Tank is a random factor. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[a] Log($x + 1$). [b] Square root. [c] Individual tuber sizes log($x + 1$) transformed before averaging per plant. [d] Arcsin square root.

Clones genetically determined to produce large tubers (i.e. those with large genotypic-tuber-sizes) produced larger and fewer tubers than clones with smaller genotypic-tuber-sizes (Fig. 4b,c). Vegetative biomass and the shoot-to-root ratio were both significantly affected by maternal-tuber-size, but not by genotypic-tuber-size (Table 2). Larger maternal tubers produced more vegetative biomass and had a lower allocation to shoots (i.e. a lower shoot-to-root ratio). Asexual reproductive allocation, on the other hand, increased significantly with increasing genotypic-tuber-size but was not affected by maternal-tuber-size (Table 2).

The path analysis revealed that genotypic-tuber-size had a fairly strong direct effect on tuber size (effect strength = 0.40) and a weak indirect effect, mediated by tuber production (effect strength = 0.11; Fig. 5). In contrast, maternal-tuber-size had a strong indirect effect on tuber size, mediated by vegetative biomass and/or tuber production (effect strength = 0.47) but a non-significant direct effect (Fig. 5). While genotypic-tuber-size had a direct effect on tuber size, genotypic- and maternal-tuber-size had only indirect effects on tuber number (Fig. 5). Tuber production had a strong positive effect on both tuber number and size, which were negatively correlated (Fig. 5).

DISCUSSION

Maternal-tuber-size had a large effect on produced tuber size. The magnitude of the effect, however, differed among clones. Genotypic-tuber-size did not account for the significant among-clone differences in response to maternal-tuber-size (i.e. for the variation in the slope of the clonal reaction norms): the relationship between genotypic-tuber-size and newly produced tuber size showed parallel responses (i.e. homogeneous slopes) for both maternal-tuber-size classes. Therefore, we can conclude that (1) an additive relationship exists between the genetic and maternal non-genetic components of tuber size, and (2) there is

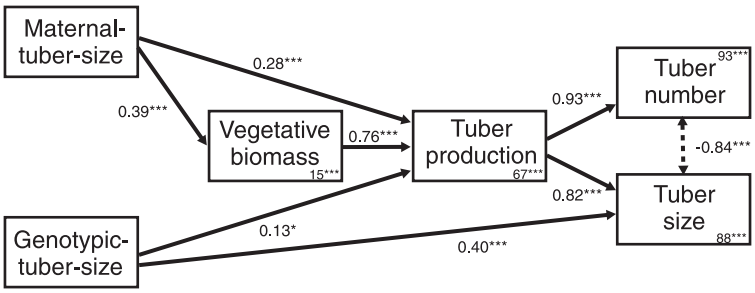

Fig. 5. Results of a path analysis for the influence of genotypic- and maternal-tuber-size on the size and number of produced tubers. All possible pathways between the four hierarchical levels in the model were tested, but only the significant relationships are depicted. Arrows indicate significant partial correlation coefficients. Solid arrows indicate positive correlations and dashed arrows negative correlations. Numbers at the arrows = partial correlation coefficients and their significance. $*P < 0.05$; $***P < 0.001$. Numbers within boxes = percentage of explained variation (adjusted R^2).

considerable clonal variation in the response of newly produced tuber size to maternal-tuber-size, which is independent of genotypic-tuber-size.

Our results show that maternal-tuber-size plays a major role in determining the tuber sizes produced. The additive relationship between genetic and maternal non-genetic effects simplifies the prediction of population responses to diversifying selection pressure observed in the field (i.e. in the population of origin of the clones), caused by spatial variation in swan predation pressure and sediment composition. However, while clones that produced large tubers were found to predominate in sandy, more heavily foraged sediment and clones producing small tubers were more abundant in clay-rich, less heavily foraged sediment (Santamaría and Rodríguez-Gironés, 2002), tuber sizes observed within the two sediment types showed a considerable range of variation. The genetic component may thus explain the differences between the two sediment types, while the maternal non-genetic effects may contribute to the increased variation observed within each sediment type. It is important to note that maternal non-genetic effects on tuber size result in an amplification of within-clone variation in tuber size across clonal generations: a given plant growing from a single tuber will produce tubers of various sizes, which will, in turn, produce plants with divergent tuber size ranges due to maternal carry-over effects. The amplification of plastic responses across generations can reduce considerably the effect of selection pressure.

Adaptive maternal effects are most likely to arise if local environmental conditions persist across generations, or more generally if the environment experienced by the mother plant is a reasonable predictor of the conditions experienced by its offspring (Rossiter, 1998; Galloway, 2001). Since tubers, in contrast to seeds, remain relatively close to the mother plant, their environment is probably similar to that of the mother plant. This may increase the strength of local selection pressure on beneficial tuber sizes, because the selective forces involved will be stable across generations.

Besides produced tuber size, maternal-tuber-size also had large effects on tuber number, total tuber production, vegetative biomass and the shoot-to-root ratio. Trait responses to maternal-tuber-size varied in all cases among clones. Genetic differences in responses to (environmental) maternal effects are not uncommon (Sultan and Bazzaz, 1993; Cheplick,

1995; Skálová *et al.*, 1997). The link between this genotypic variation and specific, quantifiable genetic traits, however, has rarely been studied. While genotypic-tuber-size did have significant effects on tuber size, tuber number and asexual reproductive allocation (and a slight effect on tuber production), this effect did not vary between maternal-tuber-sizes (non-significant genotypic-tuber-size × maternal-tuber-size interaction). Surprisingly, those traits that were affected by the genotypic-tuber-size no longer responded differently to maternal-tuber-size. Thus, as was seen for tuber size, among-clone variation in the response to maternal-tuber-size by these traits was not related to genotypic-tuber-size.

A clear distinction can be made between how maternal-tuber-size and genotypic-tuber-size affected produced tuber size. Genotypic-tuber-size predominantly showed a direct effect on produced tuber size, whereas maternal-tuber-size affected produced tuber size indirectly, by enhancing vegetative biomass and/or tuber production. Larger maternal-tuber-size also resulted in increased numbers of tubers. Whereas increasing genotypic-tuber-size resulted in decreased numbers of tubers, revealing a genetically based trade-off between tuber size and number (Fig. 4b,c). Our results may shed light on the current controversy that surrounds the identification of a hypothetical trade-off between propagule size and number (e.g. Smith and Fretwell, 1974; Maddox and Antonovics, 1983; Lloyd, 1987; Mehlman, 1993; Obeso, 1993; Wolfe, 1995; Mendez, 1997; Vaughton and Ramsey, 1998; Eriksson, 1999; Tremayne and Richards, 2000; Stuefer *et al.*, 2002). Because carry-over effects of the maternal environment (here the maternal tuber size) and genotypic effects have contrasting influences on the observed propagule size and number relationship, careful separation of these effects is required for an adequate quantification of such trade-offs (Venable, 1992). An important question in this regard concerns the regulation of the plant's reaction norm as a response to maternal effects. Plastic variation in produced tuber size may be interpreted as an adaptive, bet-hedging strategy, allowing for increased tuber size in favourable microsites (where maternal resources are abundant but competition is strong) but ensuring the production of at least some propagules (owing to their smaller size) in unfavourable microsites. Alternatively, they may be interpreted to reveal internal constraints in the production of propagules arising from, for example, meristem limitation (acting through constraints on tuber number) or internal source–sink dynamics (affecting tuber size; Sweet and Wareing, 1966; Herold, 1980).

In conclusion, tuber size in *P. pectinatus* depends on both genetic and maternal non-genetic effects. The genetic component has predominantly a direct effect on tuber size, while the maternal non-genetic effect is mediated by vegetative biomass and total tuber production. Genetic and maternal non-genetic effects are independent and show an additive relationship. The most likely consequence of this relationship for diversifying selection on tuber sizes in the field will be a reduction in the pace of selection, since more phenotypic variation exists in selected genotypes, rather than a qualitative change in the evolutionary outcome of selection itself.

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REFERENCES

Banovetz, S.J. and Scheiner, S.M. 1994. The effects of seed mass on the seed ecology of *Coreopsis lanceolata*. *Am. Midl. Nat.*, **131**: 65–74.

Black, J.N. 1958. Competition between plants of different initial seed sizes in swards of subterranean clover (*Trifolium subterraneum* L.) with particular reference to leaf area and the light microclimate. *Aust. J. Agric. Res.*, **9**: 299–318.

Casper, S.J. and Krausch, H.D. 1980. *Pteridophyta und Anthophyta*. Stuttgart: Fischer.

Cheplick, G.P. 1995. Genotypic variation and plasticity of clonal growth in relation to nutrient availability in *Amphibromus scabrivalvis*. *J. Ecol.*, **83**: 459–468.

Corner, E.J.H. 1966. *The Natural History of Palms*. London: Weidenfeld & Nicolson.

Eriksson, O. 1999. Seed size variation and its effect on germination and seedling performance in the clonal herb *Convallaria majalis*. *Acta Oecol.*, **20**: 61–66.

Galloway, L.F. 2001. Parental environmental effects on life history in the herbaceous plant *Campanula americana*. *Ecology*, **82**: 2781–2789.

Ganeshaiah, K.N. and Uma Shaanker, R. 1991. Seed size optimization in a tree *Butea monosperma*: a trade-off between seedling establishment and pod dispersal efficiency. *Oikos*, **60**: 3–6.

Hangelbroek, H.H., Ouborg, N.J., Santamaría, L. and Schwenk, K. 2002. Clonal diversity and structure within a population of the pondweed *Potamogeton pectinatus* foraged by Bewick's swans. *Mol. Ecol.*, **11**: 2137–2150.

Hangelbroek, H.H., Santamaría, L. and De Boer, T. 2003. Local adaptation of the pondweed *Potamogeton pectinatus* to contrasting substrate types mediated by changes in propagule provisioning. *J. Ecol.*, **91**: 1081–1092.

Harper, J.L., Lovell, P.H. and Moore, K.G. 1970. The shapes and sizes of seeds. *Annu. Rev. Ecol. Syst.*, **1**: 327–356.

Herold, A. 1980. Regulation of photosynthesis by sink activity: the missing link. *New Phytol.*, **86**: 131–144.

Huber, H., Stuefer, J.F. and Willems, J.H. 1996. Environmentally induced carry-over effects on seed production, germination and seedling performance in *Bunium bulbocastanum* (Apiaceae). *Flora*, **191**: 353–361.

Lloyd, D.G. 1987. Selection of offspring size at independence and other size-versus-number strategies. *Am. Nat.*, **129**: 800–817.

Maddox, G.D. and Antonovics, J. 1983. Experimental ecological genetics in *Plantago*: a structural equation approach to fitness components in *P. aristata* and *P. patagonica*. *Ecology*, **64**: 1092–1099.

Mazer, S.J. 1987. The quantitative genetics of life history and fitness in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed mass variation. *Am. Nat.*, **130**: 891–914.

Mehlman, D.W. 1993. Seed size and seed packaging variation in *Baptisia lanceolata* (Fabaceae). *Am. J. Bot.*, **80**: 735–742.

Mendez, M. 1997. Sources of variation in seed mass in *Arum italicum*. *Int. J. Plant Sci.*, **158**: 298–305.

Moegenburg, S.M. 1996. *Sabal palmetto* seed size: causes of variation, choices of predators, and consequences for seedlings. *Oecologia*, **106**: 539–543.

Montalvo, A.M. and Shaw, R.G. 1994. Quantitative genetics of sequential life-history and juvenile traits in the partially selfing perennial, *Aquilegia caerulea*. *Evolution*, **48**: 828–841.

Nolet, B.A., Langevoord, O., Bevan, R.M. *et al.* 2001. Spatial variation in tuber depletion by swans explained by differences in net intake rates. *Ecology*, **82**: 1655–1667.

Obeso, J.R. 1993. Seed mass variation in the perennial herb *Asphodelus albus*: sources of variation and position effect. *Oecologia*, **93**: 571–575.

Platenkamp, G.A.J. and Shaw, R.G. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. *Evolution*, **47**: 540–555.

Roach, D.A. and Wulff, R.D. 1987. Maternal effects in plants. *Annu. Rev. Ecol. Syst.*, **18**: 209–235.

Rodríguez-Gironés, M.A., Sandsten, H. and Santamaría, L. 2003. Asymmetric competition and the evolution of propagule size. *J. Ecol.*, **91**: 554–562.

Rossiter, M.C. 1996. Incidence and consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.*, **27**: 451–476.

Rossiter, M. 1998. The role of environmental variation in parental effects expression. In *Maternal Effects as Adaptations* (T.A. Mousseau and C.W. Fox, eds), pp. 112–134. New York: Oxford University Press.

Salisbury, E.J. 1942. *The Reproductive Capacity of Plants: Studies in Quantitative Biology.* London: G. Bell.

Santamaría, L. 2002. Selective waterfowl herbivory affects species dominance in a submerged plant community. *Arch. Hydrobiol.*, **153**: 353–365.

Santamaría, L. and Rodríguez-Gironés, M.A. 2002. Hiding from swans: optimal burial depth of sago pondweed tubers foraged by Bewick's swans. *J. Ecol.*, **90**: 303–315.

Schaal, B.A. 1980. Reproductive capacity and seed size in *Lupinus texensis*. *Am. J. Bot.*, **67**: 703–709.

Schmitt, J., Niles, J. and Wulff, R.D. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *Am. Nat.*, **139**: 451–466.

Skálová, H., Pecháèková, S., Suzuki, J. *et al.* 1997. Within population genetic differentiation in traits effecting clonal growth: *Festuca rubra* in a mountain grassland. *J. Evol. Biol.*, **10**: 383–406.

Smith, C.C. and Fretwell, S.D. 1974. The optimal balance between size and number of offspring. *Am. Nat.*, **108**: 499–506.

StatSoft, Inc. 2001. *Statistica*, version 6. Tulsa, OK: StatSoft.

Stuefer, J.F., van Hulzen, H. and During, H.J. 2002. A genotypic trade-off between the number and size of clonal offspring in the stoloniferous herb *Potentilla reptans*. *J. Evol. Biol.*, **15**: 880–884.

Sultan, S.E. and Bazzaz, F.A. 1993. Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. *Evolution*, **47**: 1009–1031.

Susko, D.J. and Lovett-Doust, L. 2000. Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). *Am. J. Bot.*, **87**: 56–66.

Sweet, W.G. and Wareing, P.F. 1966. Role of plant growth in regulating photosynthesis. *Nature*, **210**: 77–79.

Tremayne, M.A. and Richards, A.J. 2000. Seed weight and seed number affect subsequent fitness in outcrossing and selfing *Primula* species. *New Phytol.*, **148**: 127–142.

Van Groenendael, J.M. and Habekotté, B. 1988. *Cyperus esculentus* L. biology, population dynamics, and possibilities to control this neophyte. *Zeitschrift für PflanzenKrankheiten, PflanzenPathologie und PflanzenSchutz*, **XI**: 61–69.

Van Wijk, R.J. 1989. Ecological studies on *Potamogeton pectinatus* L. 3. Reproductive strategies and germination ecology. *Aquat. Bot.*, **33**: 271–299.

Vaughton, G. and Ramsey, M. 1998. Sources and consequences of seed mass variation in *Banksia marginata* (Proteaceae). *J. Ecol.*, **86**: 563–573.

Venable, D.L. 1992. Size–number trade-offs and the variation of seed size with plant resource status. *Am. Nat.*, **140**: 287–304.

Vera, M.L. 1997. Effects of altitude and seed size on germination and seedling survival of heathland plants in North Spain. *Plant Ecol.*, **133**: 101–106.

Vos, P., Hogers, R., Bleeker, M. *et al.* 1995. AFLP: a new technique for DNA-fingerprinting. *Nucleic Acids Res.*, **23**: 4407–4414.

Weiner, J., Martinez, S., MullerScharer, H., Stoll, P. and Schmid, B. 1997. How important are environmental maternal effects in plants? A study with *Centaurea maculosa*. *J. Ecol.*, **85**: 133–142.

Wiegleb, G. and Kaplan, Z. 1998. An account of the species of *Potamogeton* L. (Potamogetonaceae). *Folia Geobot.*, **33**: 241–316.

Wolfe, L.M. 1995. The genetics and ecology of seed size variation in a biennial plant, *Hydrophyllum appendiculatum* (Hydrophyllaceae). *Oecologia*, **101**: 343–352.

Wulff, R.A. 1986. Seed size variation in *Desmodium paniculatum*. III. Effects of reproductive yield and competitive ability. *J. Ecol.*, **74**: 115–121.