# Allocation among reproductive tissues is coordinated with seed size

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# Abstract

To produce viable seeds, plants allocate mass to a wide variety of other reproductive tissues; yet little is known about the relative magnitude of these investments. We quantified overall reproductive investment and its partitioning among different reproductive tissues for 14 perennial species in a recurrent-fire coastal heath community. Integrating ideas from two lineages of evolutionary theory, we found support for our overall prediction that the relative investment in different reproductive tissues will be correlated with a species’ seed size. More specifically, species with larger seeds were found to mature a smaller proportion of ovules, expend more of pre-pollination investment on discarded tissues, and invest more in seed provisioning compared to pollen attraction. We also found that the total cost of accessory tissues – i.e. mass not directly invested in the seed – was very high, varying from 95.8% to 99.8% of total investment for species in this study. This suggests that studies only counting seeds substantially underestimate total reproductive investment A species’ seed size positions it along a colonization-competition life-history spectrum; here we have shown how relative investment in pollen-attraction versus provisioning tissues, and successful versus discarded buds and ovules also form part of this spectrum, thereby contributing to the diversity in the relatives sizes of floral and fruiting structures among angiosperms.

# Introduction

Plants allocate a sizeable share of their photosynthetic energy to reproduction (Obeso 2004; Hirayama *et al.* 2008; Thomas 2011; Wenk & Falster 2015). This allocation takes the form of provisioned seeds and also of many other tissues associated with reproduction, termed accessory costs. Accessory costs include mass associated with forming a successful seed (e.g. flower petals, seed pod, and dispersal tissues) and mass lost via aborted and discarded buds, flowers and fruit. Previous studies show that for perennial species anywhere from 15% – 99% of total reproductive investment may go into accessory costs (Haig & Westoby 1988; Ashman 1994; Henery & Westoby 2001; Lord & Westoby 2006; Chen, Felker & Sun 2010). Since fruit set and seed set are generally below 50% in perennial species (Stephenson 1981; Wiens 1984; Sutherland 1986; Knight *et al.* 2005; Rosenheim *et al.* 2014), the cost of aborted and discarded tissues may be a substantial proportion of total accessory costs. Yet, little is known about the allocation of mass among different reproductive tissues across the plant kingdom and how this links with plant reproductive strategies.

Although plant species demonstrate an extraordinary diversity of reproductive structures and strategies, reproductive investment can be divided into some general functional categories (Fig. 1) that are broadly consistent among species with similar reproductive systems. In this analysis we restrict our attention to woody angiosperms, and consider investment only from the perspective of a maternal plant (Fig. 1). The total mass a mother invests in reproduction includes investment in buds and ovules that continue to develop into mature propagules, and investment in buds and ovules that fail or are voluntarily discarded before maturing fully. Considering propagules that successfully mature, mass is invested in structures whose primary goal is to attract pollen and pollinators (*pollen-attraction costs*; i.e. the flower, including petals, calyx, pedicel) and structures that develop post-pollination in maturing the propagule (*provisioning costs*). These provisioning costs comprise the *seed* itself and *dispersal and packaging* tissues. Total *accessory costs* are then all tissues besides the seed mass itself,including costs of discarded tissue, pollen-attraction, packaging and dispersal.

There are multiple reasons to expect that accessory costs will be not only large, but larger than the mass of the seed itself. Pollen-attraction is undeniably beneficial for successful formation and dispersal of a seed. Without showy petals insects would not be attracted to the stamens and stigma, without sepals the developing bud would not be protected, without a seed coat a seed would not be protected during dispersal, and without an attractive fruit, many seeds would not be dispersed. The cost of failed or discarded tissue (pre- or post-pollination) is often high in perennial plants for a diversity of reasons, some the result of conditions beyond the plant’s control and others by evolutionary design to increase fitness. The latter mechanisms include pollen-limitation, pollen-ovule incompatibility, parental embryo abortion, resource limitation and bet-hedging strategies to capitalize on stochastic variation in pollen availability, pollen quality, and resource availability to mature fertilized ovules (Bierzychudek 1981; Stephenson 1981; Sutherland 1986; Burd 1994, 2008; Ramsey 1997; Obeso 2004; Ashman *et al.* 2004; Knight *et al.* 2005; Holland & Chamberlain 2007; Rosenheim, Schreiber & Williams 2015).

The question we address in this study is whether there is systematic variation among species in how mass devoted to reproduction is distributed among the different reproductive tissues displayed in Fig. 1. In particular, we propose and test hypotheses that the relative amounts invested in different reproductive tissues are coordinated with a species’ seed size. These hypotheses arise from integrating two existing and well established trade-offs. The first is the well-supported seed size-seed number trade-off, from the plant functional trait literature. The second is the seed set-pollen-attraction cost trade-off, described in the maternal optimist-pessimist literature.

The seed size – seed number trade-off simply states that whatever mass a plant has available for seed production can be divided into many small seeds or fewer larger seeds (Smith & Fretwell 1974). This trade-off logically applies within an individual plant, as a result of basic conservation of matter. A negative correlation between seed size and number can also be observed among species, when seed count is normalised by the total amount of reproductive investment or leaf area (Henery & Westoby 2001; Moles *et al.* 2004; Sadras 2007). Within angiosperms, seed size varies XXX orders of magnitude, with very small and very large seeds as endpoints of a continuous spectrum of life history strategies (Rees & Westoby 1997; Leishman 2001; Turnbull *et al.* 2004; Moles & Westoby 2006).

The second trade-off considers the amount invested in pollen attraction versus seed provisioning. Within an individual plant, conservation of mass again implies there is an inevitable trade-off between these two activities (Haig & Westoby 1988, Rosenheim *et al.* 2014, 2016).. Based on this trade-off, Haig & Westoby (1988) argue that plants 1) produce excess ovules and flowers to optimize seed production across a population and across time, 2) allocate just enough to pollen-attracting tissues to ensure pollination of the number of ovules they are able to provision *on average*. This initial model has been extended to show that species with relatively low pollen-attraction costs should produce a greater excess of ovules relative to what they are able to provision; leading to lower seedset (the ratio of seed count to ovule count) (Rosenheim *et al.* 2014, 2016). One way of describing a species with low average seedset is as a maternal optimist. Such a species is “optimistic” in the sense that should environmental conditions be unusually favorable, it will be able to respond with increased seed production. To make this possible, an optimist discards many ovules – both pollinated and unpollinated – in most years. As it does not matter if all of its ovules aren’t pollinated, optimists are also invest proportionately less in pollen attraction. Having a low seedset also offers the optimist more opportunity to selectively choose which zygotes to provision termed selective abortion (Willson & Burley 1983; Sutherland 1986; Kozlowski & Stearns 1989; Guittian 1993; Melser & Klinkhamer 2001; Harder & Barrett 2006). Although low seedset can arise via a number of processes that occur between ovule maturation and the onset of zygote provisioning, including pollen-limitation, pollen-ovule incompatibility; selective embryo abortion is expected to be stronger in species with a relatively higher ovule count, i.e. maternal optimists.

While each of these two theory lineages is quite developed, their interaction have not previously been considered. Yet there are reasons to expect the two tradeoffs to become entangled and mutually reinforce each other. We predict that at one end of the spectrum are species that produce relatively few, but large seeds, and have low seedset. Such maternal optimists would display greater selectivity in which zygotes to provision, since they are investing more mass in each offspring and maturing fewer seeds; and invest relatively more in seed provisioning and relatively less in pollen attraction per ovule. By contrast, maternal pessimists would produce relatively fewer, more costly ovules and relatively more, less costly seeds. Two previous studies have indeed observed that big-seeded species have lower seedset (Lord & Westoby 2006, 2012).

Based on these trade-offs we predict that the proportion of reproductive mass allocated among the competing tissues shown in Fig. 1 will vary with seed size such that large seeded species:

1. Have a greater ratio of ovules to seeds, since they are expected to produce many inexpensive ovules and be more choosy about which ones they continue to invest in;
2. Invest a greater fraction of pre-pollination resources into failed or discarded tissues, as most of the ovules produced will be shed or aborted before the onset of provisioning;
3. Invest a lower fraction of post-pollination resources into failed or discarded tissues, as once they begin provisioning a zygote, large seeded species are more likely to successfully create a viable seed;
4. Invest a greater proportion of resources devoted to successfully matured seeds to seed provisioning and less pollen-attraction costs, since they produce many inexpensive ovules,
5. Invest a similar proportion of resources on seed packaging and dispersal structures.

Finally, given the complexity of measuring all the components of reproductive investment and how rarely this has been attempted for multiple species, we also assess how well different surrogate measures potentially predict total reproductive investment.

# Methods

## Study system

The study was carried out in Kuring’gai National Park, a recurrent-fire coastal heath community northeast of Sydney, Australia. We selected 14 species of woody perennials that are common in the community, regenerate from seed post-fire, and have asymptotic heights ranging from 0.5–5 m (Table 1). Myrtaceae species are well represented in the community, but absent from the study, as all locally common Myrtaceae re-sprout following fire. All sites were chosen to have minimal *Eucalyptus* cover, such that *B. ericifolia*, *H. teretifolia*, and *Allocasuarina distyla* (not included in our study because it is dioecious) were the dominant canopy species late in succession, at heights of 3-5 m.

## Field measurements

The study was conducted between May 2012 - June 2013. Initial plant measurements and subsequent harvest were conducted during the late austral autumn and early winter, the period of minimal vegetative growth in this plant community. Repeat visits were made at multiple sites throughout the year to record reproductive activity. Plants were tagged during May-June 2012 and monitoring through to May-June 2013. We selected May-June as a started/end point as these months entail minimal reproductive activity. Only *B. ericifolia*, *G. speciosa,* and (occasionally) *H. purpurea* flowered during this period, although some species had immature fruit from the previous year (*P. lanceolata*) or small buds that would open in the subsequent year (*B. ledifolia*, *C. ericifolium*, *E. microphylla*, *G. buxifolia*, *L. esquamatus*).

Seven healthy individuals of each species were selected at each site. Flowering parts on all individuals were recorded during repeat surveys, every four weeks during cooler months and every three weeks during spring and summer. In each survey, all flowering parts were counted, including buds (by size class), flowers, young fruit (by size class), and mature fruit. For some species the size of immature and mature fruit and cones was also measured, as the final size of the structures was quite variable. The exact flowering parts considered varied considerably by species due to their diverse floral structures. Flowcharts detailing what flower parts were included for each species are provided in the Online Appendix - Tables S5-S18 and Figures S1-S14. The Online Appendix also includes a table that indicates how each flowering part was counted and/or measured for each species. Each of the flower parts was independently collected from multiple untagged individuals in the community to determine its dry mass.

## Calculating reproductive investment and cost components

Total reproductive investment was calculated as the sum of investment in all the different flowering parts during the year, tabulated on a dry mass basis. For each species, reproductive parts were designated as either forming up to the point of pollination (pollen-attraction; i.e. the flower) or post-pollination and were summed into one of the two respective investment pools. For floral parts that were present at the time of pollination and continued to develop into either the seed or packaging and dispersal tissues post-pollination, the fraction of the final mass present at the time of pollination was designated part of the pollen-attraction investment and the remaining fraction as part of the packaging and dispersal investment. All calculations were made on an individual basis, although the average mass of many plant parts are based on species-level measurements. These calculations yielded total pollen-attraction tissue investment and total provisioning tissue investment. Total pollen-attraction costs and total provisioning costs are calculated by dividing the respective investment values by seed count.

To calculate the three success cost components, pollen-attraction costs, packaging and dispersal costs, and seed mass, the unit mass of reproductive parts required for the successful creation and provisioning of a single propagule were summed together. For pollen-attraction tissues, unit mass was determined by dividing the mass of the part at the time of pollination by the number of ovules it supported. All calculations make the assumption that each species produces a fixed (average) number of ovules per flower, but individual-level calculations are made for cones or inflorescence stalks which support variable numbers of flowers and hence ovules. For packaging and dispersal tissues, the unit mass was calculated by dividing the mass of the part at seed maturity by the number of seeds it supported. For seed mass, we chose to designate the endosperm and embryo as the primary reproductive unit, for it provides a consistent comparison of tissue mass across species. It is hereafter referred to as *seed size*. In contrast, the propagule includes the seed coat, and additional dispersal tissues in some species, but not others. See the Online Appendix for a depiction of the parts for each species and the number of ovules in each part.

Discarded pollen-attraction tissue costs were then determined as:

Total pollen-attraction costs – Successful pollen-attraction costs.

Discarded provisioning tissue costs were then determined by the following formula, where successful provisioning costs is the sum of seed size and successful packaging and dispersal costs:

Total provisioning costs – Successful provisioning costs.

Reproductive count values used in the manuscript are defined as follows: *Ovule count* indicates the count of all ovules initiated by the plant. *Reach flowering count* indicates the count of ovules that developed to maturity and were presented to pollinators. *Post-pollen count* indicates the count of ovules that experienced at least some provisioning and is divided into *seed count*, the count of mature seeds formed, and *post-pollen aborted count,* the count of zygotes that aborted after provisioning had commenced. All counts are for a one-year time period.

Further detailed information on the calculation of all reproductive tissues is provided in the Online Appendix.

## Statistical methods

Bivariate relationships among the variables were quantified using two methods. When testing for a significant correlation between two variables we report the r2 and p-value of an general linear model. For proportional data we fit …., otherwise we assumed …

When testing whether the slope of a particular trade-off or relationship differs from a specified value, we report the slope of the Standardised Major Axis line fit to the data (Warton *et al.* 2006). All analyses were conducted in R 3.2.4 (R Core Team 2015) using the package `smatr` for comparing slopes of SMA lines (Warton *et al.* 2012). In addition, the code replicating this analysis (and all figures) is available at https://github.com/traitecoevo/reproductive\_allocation\_kuringgai (doi: will be added at proof stage).

Most analysis on species averages….

Did not consider phylogeny because….

# Results

## Accessory costs and accessory cost components

Of the 599 plants included in this study, 223 individuals produced at least one seed during the year. On average for these individuals, 97.5% of reproductive investment went to accessory tissues rather than to seeds, decreasing to 91.5% if the entire propagule mass (embryo, endosperm, dispersal tissues???).is treated as direct investment in offspring rather than just the seed (embryo and endosperm). All results reported hereafter treat embryo and endosperm as “seed size”. Among species, the proportion of mass allocated to accessory costs ranged from a low of 95.8% for *E.microphylla* to a high of 99.8% for *H. teretifolia* (Table 1). Only *B. ericifolia* and *P. pulchella* – the two cone-bearing species – invested more in successful ovules than discarded tissues (Table 1). *H. teretifolia*, *P. phylicoides*, and *P.tuberculata* spent more than 90% of their reproductive investment on discarded tissues (Table 1). For most species, discarded tissues were predominantly pre-provisioning, with aborted seeds and fruit a minor component of discarded tissue costs (Table 1). Note that fruit that aborted after flower formation but before any visible provisioning had occurred were recorded as discarded tissues, such that pollen-attraction costs included costs associated with ovules aborted due both to lack of pollination and early maternal selection.

The total amount invested in maturing successful ovules includes pollen-attraction costs plus provisioning costs. The relative sizes of these cost components varied extensively among species (Table 1). Pollen-attraction costs provisioning costs or four species, but were < 10% for 5 species (Table 1). The percentage of costs invested in provisioning tissues ranged from 18% (*E. microphylla*) to 99% (*B. ericifolia*) (Table 1). The maximum percentages of reproductive investment invested directly in seeds by any species were 4.2% for *E. microphylla* and 4.1% for *H. purpurea*.

## Changes in relative mass investment with seed size

Add result about Seedset being lower for large seeded species (Fig 3a).

The strong trade-offs between the cost to produce a specific reproductive tissue and the number of units produced by the plant is manifested as shifts in the proportion of reproductive mass invested in different reproductive tissue pools across the seed size spectrum. As seed size increases, there is also a trend toward increasing expenditure on discarded pollen-attraction tissues in comparison to successful pollen-attraction tissues (Fig. 3c; r2= 0.60, p=0.0012), reflecting the increased choosiness (decreased seed set) in larger-seeded species (r2 =0 .59 for the seed set-seed size regression; p = 0.0013). Increased seed size was only marginally related to a shift in the proportion of provisioning mass invested in successful versus discarded tissues, with larger-seeded species showing a slight increase in proportional investment in successful tissues (Fig. 3d; r2= 0.24, p=0.0741). Larger-seeded species expend a greater proportion of their *success costs* on provisioning tissues versus pollen-attraction tissues in comparison to smaller-seeded species (Fig. 3e; r2= 0.80, p<0.0001).

These shifts are also reflected in the relative slopes of the regression between seed size and provisioning costs and between seed size and pollen-attraction costs: provisioning costs show a steeper than isometric increase with seed size, while pollen-attraction costs show a less than isometric increase with seed size (Table 2). The per seed matured costs of most other reproductive tissue pools show slightly steeper than isometric increases with increasing seed size, indicating the costs are relatively higher for larger-seeded species (Table 2).

The values for the plotted points are listed in either Table 1 or Online Appendix Table S1.

## Predicting total reproductive investment

Of the 599 plants included in this study, 357 individuals produced buds and 223 individuals produced mature seeds. Even among the individuals that produced seeds, embryo and endosperm investment was only rather loosely correlated with total reproductive investment, both within and across species (Table 3, Fig. 4, and Online Appendix). All but one species showed a significant correlation between the two metrics, but only three species displayed an r2 above 0.80 and only eight of the species had an r2 above 0.70. Furthermore, the slopes and intercepts of the relationship differed across species with the result that the correlation between reproductive investment and propagule investment across individuals of all species had an r2 of just 0.52 (Fig. 4a, Table 3). Combined these results indicate that measures of seed production alone provide poor predictors of total reproductive investment.

To assess what approximation of reproductive investment was the best alternative to measuring total reproductive investment, we regressed additional investment categories against total reproductive investment. Measures that included only investment in tissues associated with the production of mature seeds, were inferior predictors of total reproductive investment compared to measures that included investment in discarded tissues (Table 3). In particular, the correlation (r2) between investment in all discarded tissues versus all reproductive tissues was 0.97, while the correlation between investment in all successful tissues (success costs\*seed count) versus all reproductive tissues was only 0.73. Investment in discarded tissues is a better predictor for two reasons. First, discarded tissues accounted for 73% of total reproductive investment; and second, mass investment into buds and flowers was more predictable, while further filtering processes occurred before buds become mature seeds. A composite metric, the count of buds initiated \* average flower mass, when regressed against total reproductive investment, had an r2 of 0.92, making it nearly as strong a predictor of total reproductive investment as discarded tissue investment. Twelve of the species had the same slope for the relationship and eleven of the species had the same intercept for the relationship as the all-individuals regression (Online Appendix Table S3).

# Discussion

There were four key outcomes from this study. First, we observed that plants of the14 long-lived perennial species studied expended a very large proportion of reproductive mass on accessory costs. Investment in seed dry mass represented a quite modest proportion of total reproductive investment (RE) for the 14 perennial species included in this study, with just 0.2-4% of RE going to seeds versus other reproductive tissues (Table 1). Even the individual with the lowest accessory costs invested just 9.5% of its RE into the seed itself. Second, we observed a trade-offs between ovule count and pollen-attraction costs and between seed count and total reproductive costs. The trade-offs indicate there exists a fixed pool of mass to invest and species differ in the relative cost of a part versus the number of parts they can produce. We also observed a trade-off between choosiness, the inverse of seedset, and pollen-attraction costs: species that expend less mass to produce an ovule produce a greater excess of ovules. These are species at the *maternal* *optimist* end of the optimist-pessimist spectrum, which have proportionally costlier provisioning tissues relative to pollen attraction tissues (Rosenheim *et al.* 2014). In combination, these trade-offs lead to systematic differences in the way reproductive mass is allocated across species, resulting in a syndrome of reproductive traits values observed for large-seeded versus small-seeded species, our third outcome. The *maternal optimists* were, as predicted, the large-seeded species: part of the big seed-size, low seedset strategy is to invest proportionally less in flower construction to the point of pollination and proportionally more in provisioning tissues. The fourth major result was that for perennial species with low seedset, total reproductive investment was best predicted by mass expenditure in buds and flowers, not by investment in seeds or fruit.

## Accessory costs are large

All species in this study allocated an enormous proportion of RE to accessory costs, both accessory success costs and discarded tissues (Fig. 1a, Table 1). Many estimates of plant mass investment in reproduction do not account for total accessory costs, leading to potentially misleading results (reviewed in Obeso 2002; Lord & Westoby 2006; Rosenheim et al. 2014; Wenk & Falster 2015). For example, studies seeking to estimate the cost of reproduction may reach erroneous conclusions if they record only shifts in seed production year upon year, ignoring investment in accessory tissues (Obeso 2002). Reproductive allocation, the proportion of mass spent on reproduction rather than on growing and replacing vegetative tissue (Ashman 1994; Bazzaz, Ackerly & Reekie 2000; Wenk & Falster 2015), will also be substantially underestimated, leading to overestimates of the proportion of mass (and absolute amount of mass) available for vegetative growth. The current study indicates that fair assessment of RE needs to account for all pools of accessory tissues, since both discarded tissue costs and success cost components (see Fig. 1a for definitions) contributed to the high accessory costs (Table 1).

Our study species have diverse floral and fruiting structures, such that disparate tissues comprise success cost expenditures in different species (Fig. 1a, Table 1). For three species (*E. microphylla, H. purpurea,* and *P. linifolia*), the costs of producing pollen-attraction tissues (on flowers that eventually produce mature seeds) was 21-27% of total RE, while for other species it was substantially less (Table 1). The two cone-producing species, *B. ericifolia* and *P. pulchella,* had the costliest packaging and dispersal tissues, spending 71.0% and 60.5% of total RE, respectively. Other species also had high packaging and dispersal expenditure due to structures including fleshy fruit (*P. lanceolata*), woody seedpods (*Grevillea* species), and thick seedcoats (*L. esquamatus*). These are tissues that must be produced to mature each seed and their exact structures have presumably evolved to maximize seed production and survival.

Discarded tissues, those tissues associated with ovules that abort instead of developing into a mature seed, are the complement to success investment. For 12 of the 14 study species, discarded tissues accounted for more than 60% of total reproductive investment (Table 1). Only in *B. ericifolia* and *P. pulchella,* the two species with very high mass investment in woody cones,was a smaller proportion of RE attributable to discarded tissues. The majority of discarded tissue costs was due to buds and flowers that were aborted before seed provisioning became substantial (Table 1). Indeed, a large mass investment in discarded tissues has been found for all species that display low seed or fruit sets (Stephenson 1981; Sutherland 1986; Ramirez & Berry 1997; Knight *et al.* 2005).

These high accessory costs, and in particular high discarded costs, should presumably be considered a cost of sex. That is, the only reason for incurring them would be in order to create zygote genomes that conferred superior fitness, compared to zygotes created by selfing or apomixis. Having a surplus of ovules, relative to the number of offspring that can be provisioned to maturity, allows the plant to be selective about which zygotes to mature. Explanations for the abortion of a large number of mature ovules near the time of pollination include environmental stochasticity, pollen-limitation, poor pollen-tube growth, pollen incompatibility, selective abortion, and resource limitation (Ashman *et al.* 2004; Knight *et al.* 2005; Ruane, Rotzin & Congleton 2014). Additional zygotes will be lost during the provisioning period due to factors including insect attack and poor environmental conditions.

In the following sections we explore whether the three trade-offs that are observed predict how relative investment in different accessory cost pools shifts across species.

## Count-cost and choosiness-cost trade-offs exist

The first two trade-offs identified in the introduction describe how a given pool of mass can be divided into many small units or proportionally fewer large units. Abundant theory and empirical evidence underpins the seed size-seed number trade-off (Smith & Fretwell 1974; Moles *et al.* 2004; Sadras 2007) and here we extend the theory to include two trade-offs that account for the significant accessory costs required for seed production. The first trade-off is between seed count and total reproductive costs, closely related to the well-established seed size-seed count trade-off, demonstrating that large-seeded species are those species with high overall per seed reproductive costs and low seed counts (Smith & Fretwell 1974; Rees & Westoby 1997; Moles & Westoby 2006). The second is the ovule count-pollen-attraction costs trade-off, suggesting plants have a fixed pool of mass to allocate to construct flowers to the point of pollination and this mass may be divided into fewer showier flowers or into more numerous but cheaper flowers (Rosenheim *et al.* 2014).

The third trade-off is between choosiness (inverse of seedset) and the relative cost of producing a single ovule to the point of pollination: species for whom producing an ovule is less costly tend to have lower seedset (Lord & Westoby 2006; Rosenheim *et al.* 2014). Species with low seed set are also termed maternal optimists: they produce excess pollinated ovules, relative to the seeds they can provision in an average year, because they are always optimistic that the year will be better than average. Due to the large number of ovules they produce, they are selected to reduce their pollen-attraction costs (Haig & Westoby 1988; Schreiber *et al.* 2015; Rosenheim *et al.* 2015). Since these species have lower seed output, they are under stronger selection to produce seeds that will successfully establish (Lord & Westoby 2006). Simply being larger is part of their strategy (Moles & Westoby 2006), but ensuring their seeds have vigorous genotypes is another correlate of this same strategy dimension and one achieved through greater choosiness for the most vigorous embryos shortly after pollination (Westoby & Rice 1982; Willson & Burley 1983; Sutherland 1986; Guittian 1993). Having excess ovules pollinated means maternal optimists can be more selective in terms of pollen receipt (Zimmerman & Pyke 1988) and which zygotes to provision (Willson & Burley 1983; Sutherland 1986; Guittian 1993).

## Coordinated shifts in reproductive mass allocation across species

Together, the three trade-offs predict a single axis of variation in reproductive strategies, showing how species exhibit coordinated shifts in resource allocation, leading to a syndrome of reproductive traits associated with large-seeded versus small-seeded species (Fig. 1c). At one end of the spectrum are maternal optimists, using their pool of pre-pollination mass to produce many, inexpensive ovules, but their total pool of reproductive mass to produce relatively few, costly seeds, resulting in low seedset. The maternal pessimists fall on the opposite end of the spectrum. As a result, species are expected to be under strong selection to coordinate their relative investment in the different mass pools described in Fig. 1a. The first and third of the predicted relative shifts in tissue investment with seed size were strongly borne out by our data, while support for the second was weaker. First, since large-seeded species had lower seedset – and in particular high ovule and embryo abortion near the point of pollination – they spent a larger proportion of their pool of mass for pollen-attraction tissues on tissues that are discarded, relative to smaller-seeded species (Fig. 3c). Second, since these large-seeded species had a small proportion of ovules passing through the many filters to reach the point of provisioning and since these embryos had likely been carefully selected, the large-seeded species were expected to provision a larger proportion of the selected embryos to become mature seeds. There was only a weak trend in this direction, in part reflecting the overall high success rate of embryos once post-pollination provisioning commenced among species of all seed sizes (Fig. 3d).

Third, given that large-seeded species were producing relatively many inexpensive ovules and relatively fewer expensive seeds, the proportion of *success costs* allocated to pollen-attraction materials was expected to decrease with seed size while the proportion of *success costs* allocated to provisioning materials should increase with seed size, a pattern strongly observed among the study species (Fig. 3e). This represents a fundamental shift in floral construction with seed size. In relative terms, larger-seeded species were producing larger packaging and dispersal tissues, but less costly pollen-attraction materials. This is being accomplished both through a reduction in floral size and, for some plant families, an increase in the number of ovules per flower or inflorescence (Lord & Westoby 2006, 2012). This trend can also be depicted by plotting pollen-attraction costs and provisioning costs against seed size: *Pollen-attraction costs* display a less than isometric increase with increasing seed size, while *provisioning costs* display a greater than isometric increase with increasing seed size (Table 2). Identical patterns have been observed in other studies (Lord & Westoby 2006, 2012). They have been attributed in part to larger seeded-species tending to have biotic dispersal agents, with animal-dispersed species allocating a greater proportion of their reproductive mass to packaging and dispersal materials (Hughes *et al.* 1994; Moles *et al.* 2005; Eriksson 2008).

In this study, total reproductive costs and accessory costs both showed a steeper than isometric increase with seed size (Table 2), indicating the proportion of reproductive mass invested in accessory tissues is higher in larger-seeded species. Our result suggests that among our study species there are (slight) additional benefits to being large-seeded that have not been explored in this study, such as higher seedling germination and success (Moles & Westoby 2006). Previous studies have not found evidence for the increase in total reproductive costs and accessory costs with increasing seed size to be other than isometric in angiosperms (Henery & Westoby 2001; Moles, Warton & Westoby 2003; Lord & Westoby 2006; Chen *et al.* 2010; Lord & Westoby 2012). Note, that in these studies, seed size was defined as the mass of the entire propagule. When we recalculate the slopes of the relationships using total propagule size, we too observe an isometric relationship between total reproductive costs or total accessory costs and propagule size (Online Appendix Table S4.)

## Shifts in accessory costs with plant size and age

An additional motivation for this study was to determine if accessory costs shifted with plant age, size or RE. The theoretical literature suggests that for plants to increase their allocation to reproduction (versus growth) as they grow and age, plants must realize some compounding benefit (Myers & Doyle 1983; Sibly, Calow & Nichols 1985; Reekie & Bazzaz 1987a; Kozlowski 1992). Increasing mortality with age has the effect of decreasing future reproductive value and selecting for increased current RE in older plants. If accessory costs declined with RE, making seed production more efficient, then plants should be selected to have fewer, larger reproductive episodes (Kelly 1994; Kelly & Sork 2002) or to delay reproduction until they are larger and can invest more mass in reproduction (Cole 1954; Wenk & Falster 2015). Such a pattern was not observed in this dataset. Across individuals within a species, total accessory costs and accessory cost components barely shifted with plant size, age, or total reproductive investment (Online Appendix Table S2). The consistent lack of shift in per seed accessory costs (or seedset, data not shown) with RE (or bud count, data not shown) surprised us. There is a large literature on expected and observed trends in pollination and seedset with the size of the floral display, showing varied patterns (e.g. Primack 1987; Klinkhamer, de Jong & de Bruyn 1989; Ohara & Higashi 1994; Goulson et al. 1998), but the literature had not led us to expect a flat relationship for all 14 species (Online Appendix Table S2). For many of the species studied here sample sizes were large and we sampled across their entire age range. We believe that if a shift in accessory costs (or accessory cost components) existed with plant size, age, or RE for these species it should have been detected in this data.

## Estimating reproductive effort

Realistic estimates of RE are essential for many research questions, for example plant functional growth models require estimates of the proportion of photosynthetic mass that is allocated to growth versus reproduction (Fisher *et al.* 2010; Falster *et al.* 2011; Scheiter, Langan & Higgins 2013), while demographic models may need estimates of seed production for a given RE (Garcia & Ehrlen 2002; Miller *et al.* 2012). The current study, along with others, has shown that plants are allocating mass to many different reproductive tissues, with a notably small proportion going to seeds. However, the detailed measurements required to account for all reproductive mass expenditure are not practical for many research projects and pointing researchers to the best rapidly-obtainable estimates of total RE would be beneficial to many.

At the individual level, embryo and endosperm investment, propagule investment, and fruit investment were relatively poor predictors of RE (Table 3). Even within species, knowing seed investment provided only a mediocre estimate for total RE, with only 8 of the 14 species having an r2>0.70 and one species not even displaying a significant correlation across individuals (Online Appendix Table S3). In contrast total investment in discarded tissues (primarily representing investment in aborted flowers and buds), and our artificial composite measure “total bud count \* average flower mass at the time of pollination”, provided strong estimates of total RE (r2=0.96 and r2=0.92 respectively for regressions across all individuals; Table 3). While total discarded tissue investment is not a “quick measure”, requiring repeat visits to the field and tedious accounting, the composite measure would work well for species where most of their buds and flowers are visible at a single point in time. Doing a single bud count and determining flower mass for the species would be a manageable prospect and give you a quite accurate estimate of total RE. This composite metric has the merits that it would be relatively easy to measure on large numbers of plants and that it effectively combines both the within and across species variation (Fig. 4b, Table 3, Online Appendix Table S3).

Conversely, these results demonstrate that if your research question requires seed investment or seed count as an output, estimates of RE will not accurately predict seed production. Instead, and in contrast to many herbaceous species (Shipley & Dion 1992), for perennial species with relatively low seedset, seed count or seed investment must be determined for each individual.

The explanation for the poor correlation between seed investment and RE is clear: most of these species have relatively low seedset (Table 1) and moreover, seed set is quite variable across individuals at a single site (Fig. 4). The unpredictability of seedset and overall low seedset means that investment in seeds, at the individual level, cannot be predicted by any easy-to-measure metrics. Many stochastic processes, from pollinator activity to pollen compatibility to resource availability lie between bud production and seed production (Herrera *et al.* 1998; Wesselingh 2007; Gómez 2008). These processes lead to both individual and inter-annual variation in seed production (Copland & Whelan 1989; Mitchell 1997; Herrera *et al.* 1998).

## Methodological considerations

To reach meaningful conclusions about trade-offs between reproductive costs, counts, and seedset, accurate measurements of total reproductive investment are essential. Our accounting scheme is very detailed, but of course imperfect. The largest source of error is that we have not measured nectar production. Some of these species are known to produce abundant nectar, particularly *B. ericifolia*, *H. teretifolia* andboth *Grevillea* species (Pyke 1983; Pyke, O’Connor & Recher 1993; Lloyd, Ayre & Whelan 2002). Very rough back-of-the-envelope calculations, based on studies of closely related species in nearby communities, indicate nectar production increases total reproductive investment by ~20% for *G. speciosa*, 10% for *H. teretifolia*, and well under 5% for *G. buxifolia* and *B. ericifolia*. Accounting for nectar production in our study would have the effect of increasing pollen-attraction costs (both successful and discarded) relative to provisioning costs (Pyke 1983; Pyke *et al.* 1993; Lloyd *et al.* 2002).

Are dry masses the best measures of expenditure, especially in a community growing on soils known to be very low in P (Beadle 1968)? Previous studies indicate that using the concentration of a limiting mineral nutrient to calculate nutrient allocations may be a better measure of a plant’s allocation choices (Reekie & Bazzaz 1987b; Ashman 1994; Rosenheim *et al.* 2014), but also that all currencies yield similar results. For example, nectar production, in comparison to reproductive tissues such as seeds, might seem relatively less expensive, in units of P than in units of dry mass or mass, potentially relevant for a community growing on low P soils. This is a direction for future investigations.

A persistent issue in assessing reproductive costs is that some green reproductive tissues are known to photosynthesize (Cohen 1976; Reekie & Bazzaz 1987a; Wesselingh 2007). It can be argued that their dry mass is not a fair measure of cost, with some of it being paid back from their own photosynthesis. Against this, it can be argued that all the plant's photosynthesis should be considered a common pool of resource, and dry mass of different parts fairly reflects the relative allocation to different activities and tissue functions. We have adopted this second view.

This dataset does not address other known factors that may contribute to low seedset in this system, including pollen-limitation (Burd 2008, 2016) and environmental stochasticity. Insufficient pollen receipt may certainly be contributing to the patterns observed, but given recent theoretical treatments that suggest pollen-limitation should be more severe among maternal-pessimists (Rosenheim *et al.* 2014, 2016), it is unlikely the observed trend of lower seedset among the maternal-optimists is primarily attributable to pollen-limitation. Environmental stochasticity, both in terms of pollen receipt and resources to provision embryos, also selects for overproduction of embryos in maternal optimists (Haig & Westoby 1988; Rosenheim *et al.* 2014). Maternal optimists are so-named because they are optimistic about the number of ovules they will be able to mature and therefore produce additional ovules that can be matured when sufficient resources are available (Mock & Forbes 1995; Burd *et al.* 2009; Schreiber *et al.* 2015; Rosenheim *et al.* 2015).

## Conclusions

In summary, the correlations observed in our study indicate that seed size, ovule production versus seed production, and the magnitude of specific reproductive tissue pools are coordinated across species. While a plant’s accessory costs may be startlingly large at first glance, allocation of mass to different tissues is expected to represent an evolved strategy to maximize fitness. Identifying trade-offs between specific mass allocation choices – and then determining that mass allocation within this community matches the predicted patterns – provides a framework for understanding coordinated responses for seed size, seedset, and allocation to pollen-attraction versus seed provisioning tissues. Just as species have long been shown to follow a seed size-seed number trade-off, so do all species have the same amount of mass (relative to their leaf area) to invest in ovules, leading to a trade-off between the cost of pollen-attraction tissues and ovule count. Large-seeded, low seedset species have proportionally less costly pollen-attraction tissues and on average produce a proportionally larger excess of ovules relative to the seeds they are able to provision.

# Supplementary information

Online Appendix A) Additional results and details on methods.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | Proportion reproductive costs (%) | | | | |  | Proportion success costs (%) | |
|  |  |  |  | Discarded or failed | | Successfully matured seeds | | |  |  |  |
| species | Seed size (mg) | Seedset | Reproductive costs  (mg) | Pollen attraction | Packaging and dispersal | Pollen attraction | Packaging and dispersal | Seed | Success costs (mg) | Pollen attraction | Provisioning |
| *Epacris microphylla* | 0.014 | 0.317 | 0.525 | 54.6 | 12.3 | 27.0 | 1.9 | 4.2 | 0.112 | 81.6 | 18.4 |
| *Pimelea linifolia* | 0.183 | 0.274 | 10.463 | 64.0 | 2.1 | 23.7 | 8.1 | 2.0 | 3.05 | 70.0 | 30.0 |
| *Hemigenia purpurea* | 0.222 | 0.312 | 7.054 | 45.5 | 21.8 | 21.7 | 6.9 | 4.1 | 1.841 | 65.5 | 34.5 |
| *Leucopogon esquamatus* | 0.405 | 0.312 | 24.695 | 29.4 | 25.6 | 13.1 | 28.4 | 3.5 | 5.164 | 28.8 | 71.2 |
| *Conospermum ericifolium* | 0.589 | 0.177 | 24.020 | 43.0 | 34.5 | 11.1 | 8.7 | 2.7 | 5.078 | 52.4 | 47.6 |
| *Pultenaea tuberculata* | 0.867 | 0.067 | 121.561 | 85.1 | 5.5 | 5.9 | 2.4 | 1.2 | 6.951 | 62.8 | 37.2 |
| *Phyllota phylicoides* | 1.394 | 0.047 | 302.609 | 87.1 | 4.4 | 4.0 | 2.9 | 1.6 | 7.449 | 48.0 | 52.0 |
| *Boronia ledifolia* | 2.158 | 0.036 | 151.481 | 68.8 | 11.9 | 4.2 | 12.3 | 2.8 | 14.802 | 21.8 | 78.2 |
| *Petrophile pulchella* | 2.531 | 0.341 | 180.254 | 18.2 | 10.5 | 8.5 | 60.5 | 2.2 | 119.907 | 11.3 | 88.7 |
| *Persoonia lanceolata* | 3.368 | 0.062 | 1140.445 | 50.6 | 12.2 | 2.3 | 34.4 | 0.4 | 283.475 | 6.4 | 93.6 |
| *Hakea teretifolia* | 7.559 | 0.004 | 4348.682 | 80.0 | 14.0 | 0.3 | 5.5 | 0.2 | 205.875 | 5.6 | 94.4 |
| *Grevillea speciosa* | 7.728 | 0.014 | 587.822 | 59.4 | 19.5 | 1.6 | 17.4 | 2.0 | 83.208 | 8.0 | 92.0 |
| *Banksia ericifolia* | 18.511 | 0.032 | 3385.342 | 16.8 | 10.4 | 0.7 | 71.0 | 1.1 | 2218.899 | 0.8 | 99.2 |
| *Grevillea buxifolia* | 22.408 | 0.015 | 844.298 | 68.7 | 9.5 | 1.4 | 17.1 | 3.3 | 149.768 | 6.2 | 93.8 |

**Table 1.** Details on study species and their reproductive investment. Seed size indicates the mass of the embryo and endosperm only. Seedset is mature seeds per ovule initiated. Reproductive costs are the total reproductive investment per seed matured. The proportion of reproductive costs allocated to discarded tissues formed for pollen-attraction versus packaging and dispersal, successful pollen-attraction tissues, successful packaging and dispersal tissues and the seed itself are shown. Success costs are the components of total reproductive costs required for the formation of a successful seed, and are divided into two components, pollen attraction costs and provisioning costs. Note that for seed costs, the mass of the seed itself is considered part of provisioning costs.

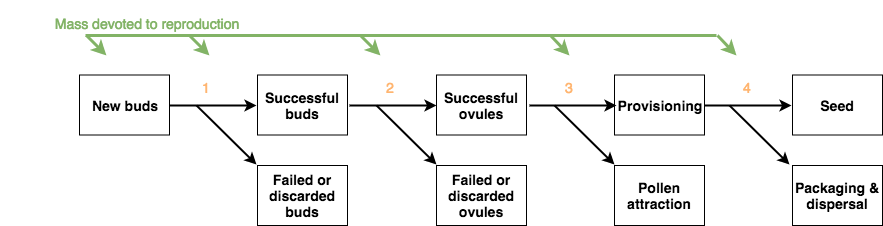
|  |  |  |
| --- | --- | --- |
| Reproductive cost component | r2 | Slope  *(confidence interval)* |
| Total reproductive costs | 0.91 | 1.27 (1.05 - 1.54) |
| Total accessory costs | 0.90 | 1.27 (1.05 - 1.54) |
| Success costs | 0.88 | 1.28 (1.02 - 1.59) |
| Pollen-attraction costs | 0.82 | 0.67 (0.52 - 0.87) |
| Provisioning costs | 0.89 | 1.51 (1.23 - 1.85) |
| Discarded tissue costs | 0.87 | 1.26 (1.01 - 1.57) |
| Discarded pollen-attraction costs | 0.85 | 1.28 (1.01 - 1.63) |
| Discarded provisioning costs | 0.87 | 1.30 (1.03 - 1.63) |

**Table 2.** Scaling of reproductive tissue costs with seed size. All variables were showed a strong correlation with seed size (p < 0.0001). Tables show properties of SMA line fits, between different variables and seed size.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Estimate of reproductive investment | | n | | r2 | |
| Total plant mass (mg) | | 357 | | 0.620 | |
| Embryo and endosperm investment (mg) | | 223 | | 0.660 | |
| Propagule investment (mg) | | 223 | | 0.525 | |
| Fruit investment (mg) | | 223 | | 0.675 | |
| Flower investment (mg)  (flower mass \* bud count) | | 223 | | 0.922 | |
| Successful investment (mg)  (success costs \* seed count) | | 223 | | 0.728 | |
| Successful pollen-attraction investment (mg) | | 223 | | 0.380 | |
| Successful provisioning investment (mg) | | 223 | | 0.736 | |
| Discarded tissues (mg) | | 357 | | 0.968 | |

**Table 3. Correlation of different estimates of reproductive investment (and total plant mass) against total reproductive investment (mg).** Regressions are done across all individuals of all 14 study species for which both reproductive investment and the *estimate* variable are greater than zero. The total cost of failed tissues or simply the mass expenditure into flowers provides the best approximation of total reproductive investment. All fits were highly significant with p < 0.0001.

# Figures



**Figure 1**. The process of producing mature seeds entails allocation among several different reproductive tissues. The total mass invested by the plant into building reproductive tissues can be partitioned into a series of four allocation points, labelled in orange. 1 – Starting at the stage of buds, mass invested can be partitioned between buds that mature successfully and those that fail to mature. 2 – Mass from the point at which flowers mature can be partitioned between ovules that mature successfully and those that fail to mature. 3 – Within ovules that mature successfully, mass invested can be partitioned between pollen attraction and seed provisioning. 4 – Within successful fruits, mass invested can be partitioned that in the seed itself and that in packaging and dispersal tissues.

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**Figure 2.** The proportion of mass allocated among different reproductive tissues for each of our 14 study species. The categories are those defined in Fig. 1. Species names are given in the Table 1.

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**Figure 3.** Relationship between a species seed size and A) Choosiness, and B-E) Fraction of mass invested between various tissues. Each point indicates an average value taken across all individuals producing reproductive tisssues for each species. Insets show the amount of variance explained and p-val for a GLM predicting Y from X.

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**Figure 4.** Comparing a poor and a good predictor of total reproductive investment. Each point indicates the reproductive investment from a single plant from the 14 study species, indicated by colors. See Table 1 for species names. Lines are best fit SMA lines for each species. There are more points in panel b, as some individuals produce buds, but no seeds. In plot b, some individual’s flower mass \* bud count is higher than their total reproductive investment due to a large proportion of buds aborting prior to reaching their mature flower mass. In this plot, propagule mass, the mass of the dispersed unit, not embryo and endosperm mass are used, as the purpose is to plot the commonly used currency.