Cone and seed trait variation in whitebark pine (*Pinus albicaulis*; Pinaceae) and the potential for phenotypic selection[1]

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Phenotypic variation among individuals is necessary for natural selection to operate and is therefore essential for adaptive evolution. However, extensive variation within individuals can mask variation among individuals and weaken the potential for selection. Here we quantify variation among and within individuals in female cone and seed traits of whitebark pine (*Pinus albicaulis*). In many plants, the production of numerous reproductive structures creates the potential for considerable variation within a plant, but these same traits should also undergo strong selection because of their direct link to plant fitness. We found about twice as much variation among individuals (overall mean = 65.3 ± 4.5% SE) than within individuals (overall mean = 34.7 ± 4.5%). One only needs to sample three to five cones per tree to accurately assess variation among trees in most cone and seed traits. The ease at which trees can be assessed helps account for the strong and consistent patterns of phenotypic selection exerted by seed predators and dispersers of whitebark pine and many other conifers. In contrast, the few traits where variation within trees equaled or exceeded that among trees underwent weak if any phenotypic selection.

Key words: phenotypic selection; phenotypic variation; Pinaceae; *Pinus albicaulis*; reproductive traits.

Natural selection drives adaptive evolution and may be responsible for most phenotypic diversification (Rieseberg et al., 2002). Because selection arises from the relationship between fitness and trait values, phenotypic variation is necessary for selection to act, with the rate of evolution limited by the amount of heritable variation among individuals (e.g., Fisher, 1930). Consequently, evolutionary biologists have devoted considerable effort to understanding the sources of phenotypic variation especially among individuals within populations (e.g., Mazer and Damuth, 2001; Hallgrímsson and Hall, 2005). However, variation within individuals can be pronounced (Willmore and Hallgrímsson, 2005), such as when an individual produces many individuals of a single organ type (e.g., flowers or fruits: Williams and Conner, 2001; Herrera et al., 2006). How variation is partitioned within individuals can be important for microevolutionary processes (Jones and German, 2005). For example, if most phenotypic variation in a trait occurs within rather than among individuals so that differences among individuals become less discrete, then the potential for selection to operate may be weakened (Williams and Conner, 2001; Herrera et al., 2006).

The size and structure of female cones and seeds of conifers are examples of reproductive traits that vary within individuals (e.g., Lyons, 1956; Smith, 1970; Elliott, 1974; Sorensen and Miles, 1978; Tomback, 1988). A number of studies have investigated how variation in cone structure and seed size affect plant fitness, and conifers have increasingly been used for inferring how diverse selection pressures influence adaptive evolution (Smith, 1970; Elliott, 1974; Coffey et al., 1999; Benkman et al., 2001, 2003; Parchman and Benkman, 2002, 2008; Siepielski and Benkman, 2004, 2007a, b). The cone structure of whitebark pine (*Pinus albicaulis*; Appendix S1, see Supplemental Data with the online version of this article), for example, has evolved in response to selection exerted by both seed predators such as pine squirrels (*Tamiasciurus* spp.) and seed dispersers such as Clark's nutcrackers (*Nucifraga columbiana*) (Siepielski and Benkman, 2007a, b). Because cone-bearing conifers produce multiple cones (often hundreds or thousands per individual), there is potential for considerable variation in cone and seed traits within trees. However, the amount of variation that occurs among individual whitebark pines and most other conifers relative to within individuals is largely unknown. The goal of our study was to quantify variation among and within trees in cone and seed traits of whitebark pine to understand whether such variation may affect the potential for phenotypic selection.

MATERIALS AND METHODS

To quantify cone and seed trait variation among and within whitebark pine trees, we haphazardly gathered 10 mature (seeds with well-developed female gametophytes) cones from 21 randomly selected trees near Union Pass in the Wind River Range, WY (43.310°N, 109.500°W) during September 2004. The mean number of cones produced per year by a whitebark pine tree over 26 years (N = 56–209 trees) in nearby Yellowstone National Park was 14, although there was considerable annual variation in the number of cones produced (Haroldson and Podruzny, 2007). Although cone size is known to vary with their location in the tree in some pines (e.g., *Pinus resinosa*; Lyons, 1956), we did not attempt to control for cone location. Because of insect damage to some cones that was not evident during collecting, an average of eight cones per tree was measured. The following cone and seed traits were measured (Appendix S1, see Supplemental Data with the online version of this article): closed cone length and maximum width, peduncle diameter, the thickness of six scales from the middle portion of the distal third and the proximal third of the cone (scales were selected approximately equidistant around the cone and were measured near their distal end), number of scales crossed by a transect along the central axis of the cone, cone mass with seeds removed, number of empty and full seeds, and for five seeds from each cone we measured kernel and seed coat masses and seed

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coat thickness (seed coat thickness was measured on the flattest surface of the seed coat) (see Benkman et al. [2003] for details of most measurements). All length measurements were made to the nearest 0.01 mm with digital calipers. All mass measurements were made to the nearest 0.1 mg with a digital scale after cones, and seeds were oven-dried at 60°–70°C for ≥2 d. One of us (R.G.) measured all cones.

We used nested analysis of variance (PROC NESTED) to estimate the percentage of variation in cone and seed traits attributed to among and within individual trees. We used analysis of variance to determine if estimates of selection exerted by nutcrackers (absolute values of selection differentials from Siepielski and Benkman, 2007a) were greater for cone and seed traits with larger ratios of among- to within-tree variation; we were unable to conduct a comparable analysis for pine squirrels because we could not estimate selection differentials (Siepielski and Benkman, 2007a). We used discriminant functions analysis (DFA) to determine the proportion of individual cones correctly assigned to the tree from which they were gathered and which traits most strongly contributed to this classification. The DFA model included the following variables: cone length, cone width, peduncle diameter, mean scale thickness, number of scales, total number of seeds, individual seed mass, and individual seed coat thickness. The cross-validation procedure was used to insure that classifications were not based on the same equations used in developing the classifications. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, North Carolina, USA).

We randomly sampled cones from each of 12 trees for which we had measured at least eight cones per tree to determine how estimates of cone and seed trait means varied with sample size. We present graphical analyses for two traits with the highest variation among trees and two traits with the highest variation within trees (excluding the number of empty seeds; discussed later). These analyses provide an estimate of how many cones need to be sampled to provide a reliable estimate of the trait means for a tree.

RESULTS

The amount of variation in cone and seed traits among trees (mean = 65.3 ± 4.5% SE) was nearly twice as large as the variation within trees (mean = 34.7 ± 4.5%; Table 1). Seed coat thickness had the largest amount of variation among trees (92%), whereas the number of empty seeds per cone had the largest amount of variation within trees (79%; Table 1). Cone width and peduncle diameter were the only other traits besides the number of empty seeds per cone where there was more variation within individuals than among individuals, albeit only slightly (Table 1). Estimates of the strength of selection (from Siepielski and Benkman 2007a) exerted by nutcrackers on

whitebark pine cone and seed traits were about three times greater for traits with ratios of among- to within-tree variation well exceeding one (≥1.68; Table 1) than for traits with ratios near to or less than one (≤1.06) (mean values [range, n] of selection differentials: 0.30 [0.11–0.52, $N = 9$] and 0.11 [0.07–0.14, $N = 4$], respectively; Welch's ANOVA: $F_{1, 9.65} = 14.28$, $P = 0.004$).

Most (mean = 83%; range = 25–100%) individual cones were correctly classified in the DFA as belonging to the tree from which they were sampled. This high level of correct classification is evident in a plot of the first two canonical function scores for individual cones, which show clusters of individual cones from the same tree (Fig. 1). Traits with more variation among than within trees were also highly correlated with the first two canonical function scores, indicating that such traits were important for distinguishing among individual trees. Seed coat thickness displayed 11.5 times more variation among than within trees (Table 1) and was strongly correlated with the first canonical score (Table 2). Similarly, the total number of seeds and individual seed mass had nearly three to four times more variation among than within trees (Table 1), and both were highly correlated with the second canonical function score (Table 2).

The number of cones that need to be sampled from a tree to provide a stable estimate of the mean varies among cone traits (Fig. 2). However, even for traits with relatively high variation within trees, such as peduncle diameter and cone width, a sample of five cones converges on the mean of larger sample sizes (Fig. 2).

DISCUSSION

Our results show that most variation in whitebark pine cone and seed traits occurs among trees rather than within them. This finding is consistent with other studies that have found greater variation among than within trees in cone and seed traits of two species of conifers (lodgepole pine, *Pinus contorta*: Smith, 1970; Elliott, 1974; Douglas-fir, *Pseudotsuga menziesii*: Sorensen and Miles, 1978). Our results are also consistent with those found for whitebark pine seed masses by Tomback (1988)

TABLE 1. Most variation in whitebark pine cone and seed traits resides among rather than within trees. Shown is the percentage of whitebark pine cone and seed trait variation explained among and within trees, the ratio of among- to within-tree variation, and the coefficient of variation (CV) among and within trees ($N = 21$ trees, with a mean of eight cones measured per tree [range = 4–10 cones]).

| Trait	Among trees	Within trees	Ratio of among to within	CV among trees	Mean CV within trees (Range)
Cone length (mm)	66.2	33.8	1.96	13.9	8.0 (2.1–14.0)
Cone width (mm)	49.1	51.1	0.96	6.4	4.7 (2.8–6.6)
Cone mass (gm)	51.5	48.5	1.06	26.5	15.1 (5.0–49.8)
Number of scales	62.7	37.3	1.68	12.7	7.6 (3.5–13.7)
Distal scale thickness (mm)	63.1	36.9	1.71	13.8	8.7 (3.9–20.9)
Distal scale mass (mg)	72.0	28.0	2.57	30.4	14.9 (7.5–53.4)
Proximal scale thickness (mm)	67.9	32.1	2.12	12.4	7.0 (3.2–16.3)
Proximal scale mass (mg)	81.3	18.7	4.35	29.4	12.3 (6.8–19.7)
Peduncle diameter (mm)	36.9	63.1	0.58	11.1	8.6 (4.6–14.8)
Total number of seeds	73.0	27.0	2.70	36.6	21.0 (7.7–38.8)
Number of full seeds	73.0	27.0	2.70	37.6	21.9 (8.7–44.9)
Number of empty seeds	21.0	79.0	0.27	89.3	74.4 (27.2–140.4)
Seed mass (mg)	81.1	19.9	4.08	19.8	8.6 (5.5–13.4)
Kernel mass (mg)	74.0	26.0	2.85	20.4	10.2 (4.5–16.2)
Seed coat mass (mg)	80.0	20.0	4.00	21.4	8.9 (4.6–14.6)
Seed coat thickness (mm)	92.0	8.0	11.50	32.4	8.1 (3.6–17.5)
Overall mean	65.3	34.8	1.88	25.9	15.0
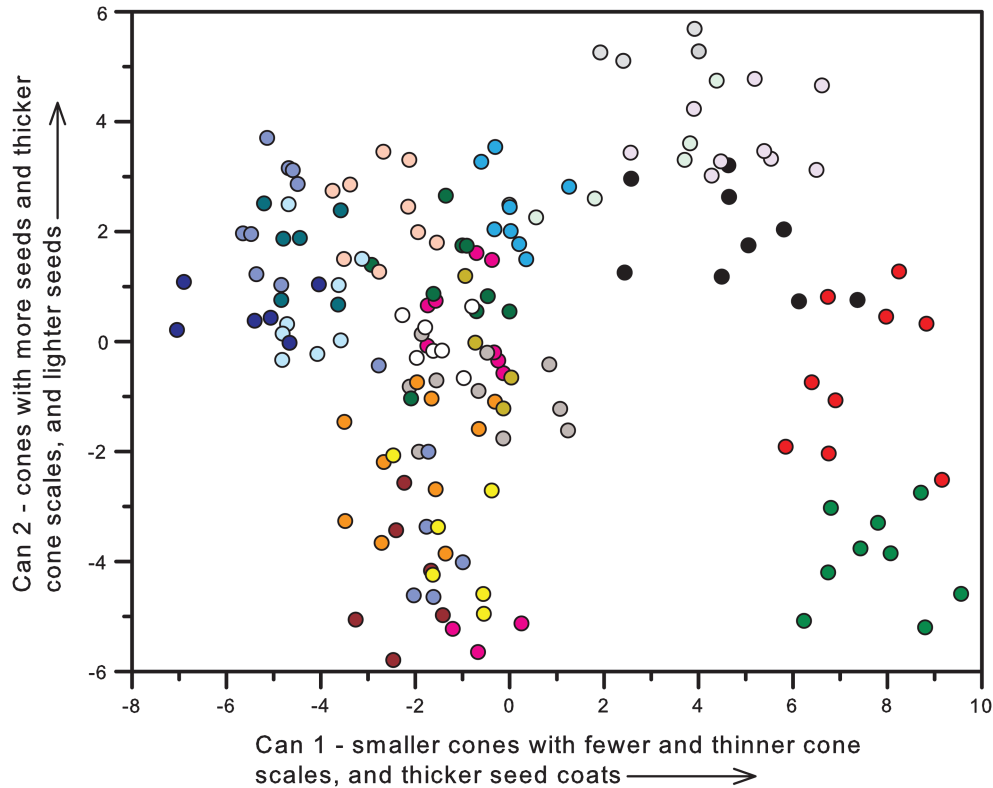
Fig. 1. Individual whitebark pine cones from a tree tend to cluster together, indicating that most variation resides among trees rather than within them. Shown are the first two canonical functions from a discriminant function analysis. Each point is an individual cone from a different tree, with each tree coded a different color.

who used the coefficient of variation (CV) to compare variation among and within trees (among-tree CV = 19.8% and 23.3% and the range of within-tree CV = 5.5–13.4% and 8.3–18.1%, respectively, for our and Tomback's studies; see Table 1).

The number of empty seeds had much higher CVs both among and within trees (Table 1; 89.3 and 74.4, respectively) than the other traits (highest other values were 37.6 and 21.9, respectively). We anticipated that the number of empty seeds would have high variation within trees because this number depends on the frequency of self-pollination (Farris and Mitton, 1984; Smith et al., 1988; Robledo-Arnuncio et al., 2004), which should be highest on the leeside and lower part of the tree (Burr et al. 2001). Variation in ambient temperature within a tree may

further contribute to variation in the frequency of empty seeds within trees (Webber et al. 2005).

Physical interference or structural constraints may also contribute to variation within trees. For example, cones growing together in clusters (online Appendix S1) may impact the development of certain traits such as cone width or peduncle diameter, which may explain why these two traits had relatively high levels of variation within trees (Table 1). Similarly, Sorensen and Miles (1978) found that the width of Douglas-fir cones varied more within than among trees. In contrast, individual seed traits (e.g., kernel mass, seed coat mass and thickness) had relatively small levels of variation within trees (Table 1). This low level of variation is perhaps related to their isolation from external factors within the developing cone.

High levels of variation among trees relative to that within trees determine the extent to which agents of selection can distinguish between individuals (Williams and Conner, 2001). For example, both pine squirrels and nutcrackers exert strong selection on the number of seeds per cone and cone scale thickness (Siepielski and Benkman, 2007a, b), two traits where variation among trees was around two to nearly three times greater than variation within trees (Table 1). In contrast, we detected only weak or no selection (Siepielski and Benkman, 2007a, b) on the two traits with the relatively largest variation within trees (peduncle diameter and number of empty seeds; Table 1). Overall, we found that estimates of selection exerted by nutcrackers were about three times greater for traits with ratios of among- to within-tree variation well exceeding one than for traits with ratios near or less than one. These findings suggest that the

TABLE 2. Spearman rank correlations between whitebark pine cone and seed traits and the first two canonical function scores from a discriminant functions analysis.

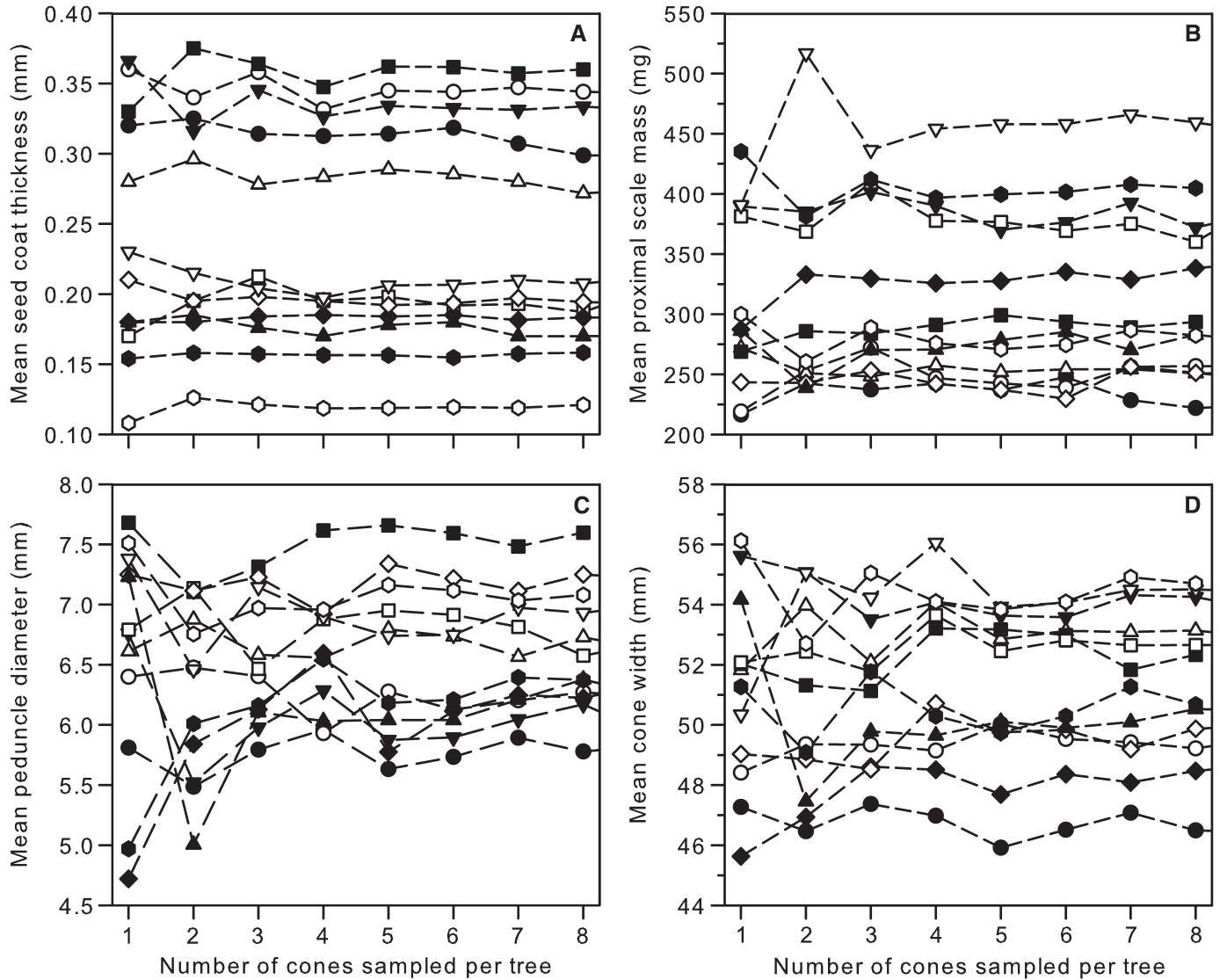| Cone trait	Can 1	Can 2
Cone length (mm)	0.275**	0.402***
Cone width (mm)	−0.119	0.080
Cone mass (gm)	−0.369***	0.036
Number of scales	−0.268**	0.284**
Mean scale thickness	−0.154*	0.526***
Peduncle diameter (mm)	−0.093	−0.179*
Total number of seeds	−0.137	0.703***
Seed mass (mg)	0.084	−0.756***
Seed coat thickness (mm)	0.955***	−0.022

Notes: $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$

Fig. 2. The mean values of four cone and seed traits in relation to the number of cones sampled from each of 12 trees for which eight or more cones were measured. Each symbol type connected by a dashed line represents the values for an individual tree, and symbol type corresponds to the same tree among figures.

strength of selection exerted on individual traits by seed predators and dispersers may be limited by the relative amount of among plant variation even when these seed consumers might benefit from discriminatory feeding.

Nevertheless, the general pattern of relatively high variation among trees compared to within trees in most cone and seed traits allows seed consumers to rapidly assess expected foraging rates on a given tree and to readily discriminate among trees. Presumably, a seed predator or disperser could gain an accurate assessment of tree quality after sampling three to five cones. In our studies, we measured either two or three cones per tree to characterize a tree's cone and seed traits and found consistent patterns of adaptive evolution of conifer cone and seed traits in response to measured selection from avian, insect, and mammalian seed predators (e.g., Benkman et al., 2001; Parchman and Benkman, 2002; Siepielski and Benkman, 2004; Mezquida and Benkman, 2005; Parchman et al., 2007; Parchman and Benkman, 2008; Siepielski and Benkman, 2008). In the future,

however, we should measure traits from at least three or four cones per tree to provide more reliable estimates of tree means.

While within individual variation in cone and seed traits appears limited among conifers, proportionately higher levels of variation within individuals have been found in some reproductive traits in herbaceous angiosperms (Williams and Conner, 2001; Herrera et al., 2006). For example, Williams and Conner (2001) found that more variation in floral traits of the wild radish (*Raphanus raphanistrum*) occurred within individuals than among individuals. Similarly, Herrera et al. (2006) found that most variation in nectar sugar composition of the insect-pollinated perennial herb *Helleborus foetidus* occurred within individuals rather than among individuals. Determining what factors contribute to the relative amounts of variation among and within individuals would increase our understanding of the potential for phenotypic selection to cause phenotypic evolution across different plant life histories (Williams and Conner,

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2001). Although data are few, perhaps we should expect relatively greater variation within individuals for smaller herbaceous plants than larger long-lived trees like conifers that can accumulate resources over longer time periods and larger areas and thus are potentially less influenced by environmental variation. In addition, cone production and development is fairly synchronous within individual whitebark (and other) pines (A. M. Siepielski and C. W. Benkman, personal observation), reducing the potential for the seasonal variation that, for example, arises within individual plants that flower repeatedly over several weeks or months (Williams and Conner, 2001; Herrera et al., 2006). Finally, some traits, such as the number of empty seeds, which is influenced by the abundance of outcrossed pollen (Smith et al. 1988), are more likely to be influenced by environmental variation and thus exhibit greater variation within individuals. Such traits would not only undergo weaker selection, but because of greater environmental variation less of the total variation would be from additive genetic variation and thus responsive to selection.

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