From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees[1]

Glenn T. Howe, Sally N. Aitken, David B. Neale, Kathleen D. Jermstad, Nicholas C. Wheeler, and Tony H.H. Chen

Abstract: Adaptation to winter cold in temperate and boreal trees involves complex genetic, physiological, and developmental processes. Genecological studies demonstrate the existence of steep genetic clines for cold adaptation traits in relation to environmental (mostly temperature related) gradients. Population differentiation is generally stronger for cold adaptation traits than for other quantitative traits and allozymes. Therefore, these traits appear to be under strong natural selection. Nonetheless, high levels of genetic variation persist within populations. The genetic control of cold adaptation traits ranges from weak to strong, with phenological traits having the highest heritabilities. Within-population genetic correlations among traits range from negligible to moderate. Generally, bud phenology and cold hardiness in the fall are genetically uncorrelated with bud phenology and cold hardiness in the spring. Analyses of quantitative trait loci indicate that cold adaptation traits are mostly controlled by multiple genes with small effects and that quantitative trait loci × environment interactions are common. Given this inherent complexity, we suggest that future research should focus on identifying and developing markers for cold adaptation candidate genes, then using multilocus, multiallelic analytical techniques to uncover the relationships between genotype and phenotype at both the individual and population levels. Ultimately, these methods may be useful for predicting the performance of genotypes in breeding programs and for better understanding the evolutionary ecology of forest trees.

Key words: association genetics, cold hardiness, dormancy, genecology, bud phenology, quantitative trait loci.

Résumé : L'adaptation au froid hivernal chez les arbres des régions tempérées et boréales implique des processus génétiques, physiologiques et ontogéniques complexes. Des études génécologiques démontrent l'existence de gradients génétiques abruptes pour les caractères d'adaptation au froid, en relation avec des gradients (surtout reliés à la température) environnementaux. Les différences entre populations sont généralement plus marquées pour les caractères d'adaptation au froid que pour les autres caractères quantitatifs incluant les allozymes. Il semble donc que ces caractères font l'objet d'une forte sélection naturelle. Cependant, on retrouve un haut degré de variation génétique au sein des populations. Le déterminisme génétique des caractères d'adaptation au froid va de faible à fort, avec les caractères phénologiques ayant les plus fortes héritabilités. À l'intérieur de la population, les corrélations génétiques entre les caractères vont de négligeables à modérées. Généralement, la phénologie des bourgeons et la résistance au froid à l'automne ne montrent pas de corrélation avec la phénologie des bourgeons et la résistance au froid au printemps. Des analyses sur les lieux des caractères quantitatifs (QTL) indiquent que les caractères d'adaptation au froid sont surtout contrôlés par des gènes multiples ayant des effets faibles et que les interactions entre les lieux des caractères quantitatifs et l'environnement sont fréquentes. Compte tenu de cette complexité inhérente, les auteurs suggèrent que les recherches futures devraient porter sur l'identification et le développement de marqueurs de gènes candidats pour l'adaptation au froid, pour ensuite utiliser des techniques d'analyses multi-lieux et multi-alléliques pour déceler les relations qui existent entre les génotypes et les phénotypes aux niveaux individuels aussi bien que des populations. Ultimement, ces méthodes pourraient s'avérer utiles pour prédire la performance de génotypes dans des programmes d'amélioration génétique, et pour mieux comprendre l'écologie évolutive de arbres forestiers.

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G.T. Howe.[2] Department of Forest Science, Oregon State University, 321 Richardson Hall, Corvallis, OR 97331-5752, U.S.A.
S.N. Aitken. Department of Forest Sciences, University of British Columbia, 3041-2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.
D.B. Neale[3] and K.D. Jermstad. Institute of Forest Genetics, Pacific Southwest Research Station, USDA – Forest Service, Placerville, CA 95667, U.S.A.
N.C. Wheeler. 21040 Flumerfelt Rd. SE, Centralia, WA 98531; Formerly, Weyerhaeuser Company, PO Box 9777, Federal Way, WA 98063, U.S.A.
T.H.H. Chen. Department of Horticulture, 4017 Agricultural and Life Sciences Building, Oregon State University, Corvallis, OR 97331-7304, U.S.A.

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[2]Corresponding author (email: Glenn.Howe@oregonstate.edu).
[3]Additional affiliation: Department of Environmental Horticulture, University of California, Davis, CA 95616, U.S.A.

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Mots clés : associations génétiques, résistance au froid, dormance, génécologie, phénologie des bourgeons, lieux des caractères quantitatifs.

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Introduction

Trees and other woody perennial plants have a remarkable ability to withstand cold temperatures. When fully acclimated, some species survive liquid nitrogen (–196 °C), yet temperatures slightly below freezing may severely damage or kill the same trees when they are actively growing (Fuchigami et al. 1971). The transition from active growth to a state of extreme cold hardiness is a protracted process that involves the coordinated expression of many traits and genes, often in response to environmental signals such as photoperiod and temperature. Many plant species respond to low temperatures, acclimate to cold, and survive temperatures slightly to moderately below freezing (Thomashow 1999). Yet, temperate and boreal woody perennial plants are distinguished by their ability to survive in regions with truly extreme winter temperatures (e.g., below –80 °C; Kramer and Kozlowski 1979). Trees and other woody perennials are also distinguished by a period of winter dormancy (endodormancy) and complex adaptations such as chilling requirements and flushing requirements that control the timing of regrowth in the spring once temperatures are again suitable for growth.

Although we know a great deal about the physiological genetics, genecology (ecological genetics), and quantitative genetics of cold adaptation in trees, the genes that underlie these traits are largely unknown. Which genes play important functional roles in cold adaptation? Which genes are responsible for the variation we observe within populations? Are these the same genes that are responsible for the differences among populations and species? Which evolutionary forces are responsible for these patterns of genetic variation? Advances in molecular genetics and genomics provide new tools to dissect the genetics of cold adaptation. Thus, we are on the verge of answering these important questions. This information will be valuable for breeding programs, gene conservation, understanding local adaptation and microevolution, and predicting the effects of global climate change.

The goals of this paper are to: (*i*) review our knowledge of the genetics of cold adaptation in forest trees; (*ii*) summarize implications for forest gene resource management including breeding, marker-assisted selection, and ecological genetics; and (*iii*) explore new approaches for unraveling the complexities of cold adaptation by applying genomics approaches to better understand the genes that vary within and among species. We will mostly draw on our experience with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and *Populus* to illustrate our conclusions.

Cold adaptation involves the coordinated expression of many component traits

In temperate and boreal regions, the annual growth cycle of woody perennials is synchronized with the annual climatic cycle. Trees alternate between periods of active growth and winter dormancy (endodormancy; Lang 1987), thereby taking advantage of favorable climatic conditions during the growing season, while avoiding cold damage during the winter. The annual growth cycle consists of many traits that are genetically and temporally correlated with cold hardiness, including: (*i*) growth cessation and bud set; (*ii*) initiation of cold acclimation; (*iii*) development of endodormancy; (*iv*) development of maximum cold hardiness; (*v*) endodormancy release via chilling; (*vi*) loss of cold hardiness; and (vii) initiation of primary growth and vegetative bud flush. We use "cold adaptation traits" to refer to these and other traits that are associated with cold hardiness.

The risk of cold injury is highest when trees are actively growing and frosts are likely. Therefore, most damage results from late spring frosts around the time of bud flush, or early fall frosts around the time of growth cessation (Timmis et al. 1994; Cannell and Smith 1984; Cannell et al. 1985*a*, 1985*b*). Frost injury models suggest that spring frosts present the greatest risk in most locations (Timmis et al. 1994). Most trees develop levels of midwinter hardiness that far exceed the recorded minimum winter temperatures (e.g., Fuchigami et al. 1971; Aitken and Adams 1996), and midwinter cold injury more likely results from winter drought than from freezing injury per se (Sakai and Larcher 1987).

Despite the wide use of bud set and bud flush as useful markers of the annual developmental cycle, growth initiation and growth cessation are only loosely correlated with these easily observable developmental stages. In Douglas-fir, for example, the renewal of mitotic activity begins as early as February (Fielder and Owens 1989), bud swelling continues through March, and bud flush may not occur until April, or even May. Nonetheless, because mitotic activity is difficult to measure, and bud flush is well correlated with spring frost hardiness, the timing of bud flush is often used as an indicator of adaptability.

The correlation between bud set and the cessation of mitotic activity is even lower, particularly for older trees. The initiation of bud scales in young seedlings may begin in midsummer, and is only the first step in the process of growth cessation that may take 3 months (Fielder and Owens 1989). Shoot elongation may continue during bud scale initiation because of continued activity of the subapical meristem and cell elongation. Destructive sampling of buds is necessary to determine when the transition from bud scale initiation to embryonic shoot formation occurs. Nonetheless, the development of cold hardiness in Douglas-fir parallels this transition and varies among breeding zones.

Within species, there is relatively little variation in cold hardiness during the active growth period (e.g., *Pinus sylvestris*, Hurme et al. 1997; *Betula pendula*, Li et al. 2003*b*). Most temperate and boreal species suffer cold injury if temperatures drop more than a few degrees below freezing between the time of bud flush (or growth initiation) and the cessation of elongation (Sakai and Larcher 1987). Because frosts are uncommon during the growing season, and because there is little variation in cold hardiness when trees are actively growing, most studies have focused on cold hardi-

ness just before elongation stops in the fall until shortly after elongation resumes in the spring.

Environmental control of cold adaptation

Cold hardiness is correlated with patterns of seasonal growth, because growth cessation is a prerequisite for the development of substantial cold hardiness (Weiser 1970). Woody perennial plants from temperate and boreal regions exhibit two major classes of elongation growth. In seasonally indeterminate species and developmental stages, preexisting and neo-formed stem units are elongated in the same growing season, primary growth (i.e., elongation growth) often occurs late into the growing season (via one or more flushes), and growth cessation (accompanied by bud set or shoot tip abscission) typically occurs in response to long nights (i.e., short days) and low night temperatures (reviewed in Barnes et al. 1998). This pattern of primary growth seems to be prominent in young seedlings, early successional species, and species that are unlikely to experience late summer droughts and frosts (reviewed in Barnes et al. 1998).

In seasonally determinate species and developmental stages, elongation growth typically stops in early summer, after the elongation of pre-existing stem units is complete. Following bud set, the formation of new stem units continues in terminal buds, but these stem units are elongated during the next growing season. The timing of bud set in these species is mostly determined by the number of stem units in terminal buds and by the period required for their elongation and maturation, rather than by current environmental conditions (Chuine et al. 2001).

Three stages of cold acclimation have been described in woody plants (Weiser 1970). The first stage of cold acclimation occurs in response to long nights (Weiser 1970), although low night temperatures may enhance growth cessation (Howe et al. 1999). The second stage of cold acclimation is largely dependent on exposure to low temperatures, and frosts appear to be an important stimulus in nature (Weiser 1970). In some species, a third stage of acclimation may occur in response to very low temperatures (–30 to –50 °C; Weiser 1970).

Endodormancy is also induced by long nights and low night temperatures (Howe et al. 1999). Nonetheless, cold hardiness can develop in the absence of dormancy (e.g., *Thuja plicata*, *Tsuga heterophylla*; Silim and Lavender 1994), and dormancy can develop in the absence of frost hardiness (Kramer and Kozlowki 1979). Endodormancy is released via chilling, i.e., by prolonged exposure to temperatures slightly above freezing (reviewed in Howe et al. 1999). Once the chilling requirement has been satisfied, cold deacclimation, the initiation of primary growth, and vegetative bud flush typically occur in response to warm temperatures (Sakai and Larcher 1987; Bigras et al. 2001). The required threshold temperature and duration of warm temperatures varies both within and among species (Chuine et al. 2001).

Although the primary environmental cues regulating the annual developmental cycle are photoperiod and temperature, other factors such as soil moisture and nutrient availability interact with these cues (e.g., van den Driessche 1969; Campbell and Sugano 1979; Silim and Lavender 1994; Bigras et al. 2001). The availability of mineral nutrients has an unclear effect on cold hardiness; fertilization can increase hardiness, decrease hardiness, or have no effect (Bigras et al. 2001). Furthermore, low night temperatures (i.e., repeated diurnal temperature fluctuations) probably activate different signaling pathways than do the one-time, short-term exposures to low temperatures that induce cold acclimation (e.g., Thomashow 2001) and the very long-term exposures that result in endodormancy release via chilling (Howe et al. 1999).

Populations of forest trees and genotypes within populations differ in their responses to the environmental cues that regulate the annual developmental cycle. Genetic differences in critical night length for bud set and dormancy induction (e.g., Eriksson et al. 1978; Howe et al. 1995; Clapham et al. 2001), chilling requirements (Campbell and Sugano 1979), and temperature requirements for bud break and initiation of growth (Chuine et al. 2001) have been documented. Genetic variation in cold hardiness per se is discussed in more detail below.

Developmental control of cold adaptation

Trees are most vulnerable to cold injury at the seedling stage. One reason is their size; seedlings are short and their meristems are in the zone of cold air that pools just above the soil surface. Second, when located in forest openings, seedlings are more likely to be damaged by radiation frosts. Finally, seedlings in their first growing season typically display seasonally indeterminate growth and grow longer into the fall when frosts are more likely to occur than do older trees. Seedlings display seasonally indeterminate growth via "free growth" or "second flushing". Free growth is the simultaneous formation and elongation of new stem units without the formation of a terminal bud, whereas "second flushing" (lammas growth) occurs when terminal buds are formed, but regrowth occurs in the same growing season without an intervening period of dormancy. First-year seedlings often display free growth throughout the middle and late summer, whereas older seedlings and saplings often second flush when adequate moisture and nutrients are available. The propensity for second flushing is genetic, but this trait is only expressed on some sites in some years (Adams and Bastien 1994; Schermann et al. 1997). Trees that second flush have a higher risk of fall cold injury than those that do not (Anekonda et al. 1998), and have a higher frequency of stem defects, including forking (Adams and Bastien 1994; Schermann et al. 1997).

In Douglas-fir, the growth of older trees is almost exclusively seasonally determinate. Presumably, the "risky" indeterminate growth habit of seedlings (via free growth and lammas growth) promotes survival during the highly competitive establishment phase, as long as the chance of frost is low in any one year. Once established, trees tend to complete elongation growth early in the season, thereby consistently avoiding damage when early frosts do occur.

Cold adaptation at the molecular level

Progress at understanding cold adaptation at the molecular level has been reviewed for trees (Faust et al. 1997; Howe et al. 1999; Rohde et al. 2000; Clapham et al. 2001) and other plants (Thomashow 1999, 2001). We are learning a great deal about the genes that regulate elongation growth, cold acclimation, and endodormancy, as well as the genes that

may directly confer cold hardiness and maintain dormancy. For example, photoperiod detection, growth cessation, and dormancy induction have been characterized at the molecular level in *Populus* (Howe et al. 1999; Rohde et al. 2000; Frewen et al. 2000), *Picea abies* (Clapham et al. 2001), and *Betula pendula* (Li et al. 2003*a*, 2003*b*). Photoreceptor proteins called phytochromes play key roles in detecting photoperiod, and the genes producing these proteins have been investigated in *Populus*, *Picea abies*, and *Pinus sylvestris* (Howe et al. 1995, 1998; Olsen et al. 1997*b*; Clapham et al. 2001). Components of phytohormone signaling pathways (e.g., abscisic acid, gibberellin, and auxin) are being studied in *Salix* (Olsen et al. 1995, 1997*a*), *Populus* (Rohde et al. 2000; Frewen et al. 2000; Eriksson and Moritz 2002), and *Betula* (Li et al. 2003*a*). Transcription factors involved in cold acclimation (e.g., CBF/DREB1) are being studied in *Populus* (Thomashow 2001; T.H.H. Chen, M.F. Tomashow, P.M. Hayes, E. Stockinger, and S. Rhee, unpublished data), as well as genes that may directly confer cold hardiness, including dehydrins in *Prunus*, *Betula*, and *Picea* (Wisniewski et al. 1999; Rinne et al. 1999; Richard et al. 2000). These examples provide only a glimpse of the large number of cold adaptation candidate genes that will be available for making the link between genotype and phenotype.

Genetic variation in cold adaptation

Genecology

Populations of forest trees are often well differentiated for cold adaptation traits

For over two centuries, botanists and foresters have recognized that variation in cold adaptation exists among populations of forest trees (Langlet 1971). Seedling genecological studies and field provenance trials reveal significant genetic variation among populations and steep genetic clines along environmental gradients for cold adaptation traits, including the timing of growth initiation and cessation, dormancy, and cold hardiness (reviewed in Morgenstern 1996). These clines are repeated in different portions of a species' range and in different species, providing strong evidence that these patterns were forged by natural selection (Endler 1977).

Population genetic studies of trees reveal relatively low differentiation among populations for selectively neutral genetic markers such as allozymes. F_{st} is a measure of the proportion of total genetic variation that is attributed to differences among populations. Commonly used estimators of F_{st} include G_{st}, θ, and R_{st} (reviewed in Merilä and Crnokrak 2001). Although F_{st} averages only 0.073 for gymnosperms and 0.084 for long-lived woody perennials (Hamrick et al. 1992), tree populations are often well differentiated for quantitative traits, particularly for traits related to the synchronization of growth and dormancy to local climates. The analog to F_{st} for quantitative traits is Q_{st}. Q_{st} estimates the proportion of total genetic variation for quantitative traits that is found among populations (Prout and Barker 1993; Spitze 1993). It can be estimated from common garden experiments that include both populations (e.g., provenances) and families within populations (e.g., full-sib, half-sib, or open-pollinated progenies):

$$[1] \quad Q_{st} = \sigma_p^2 / [\sigma_p^2 + 2\sigma_{w(p)}^2]$$

where σ_p^2 is the additive genetic variance among populations, and $\sigma_{w(p)}^2$ is the additive genetic variance within populations. Because most common garden experiments with provenance and progeny structure use open-pollinated families (i.e., seed collected from individual wind-pollinated mother trees), and because the genetic relatedness of these families approximates half-sibs, $\sigma_{w(p)}^2$ is often estimated as $4*\sigma_{f(p)}^2$, where $\sigma_{f(p)}^2$ is the variance component for open-pollinated families within populations. Using this approach, Q_{st} is slightly underestimated, because open-pollinated families are not true half-sibs, but contain a mixture of half-sibs, full-sibs, and a small proportion of seeds from self-pollination or consanguineous matings (Campbell 1979). Variation among clones within populations can also be used to estimate $\sigma_{w(p)}^2$, but this will also underestimate Q_{st}, because clonal differences include nonadditive genetic variation and nongenetic clonal ("C") effects. The resulting estimates should be reasonable, however, because most of the genetic variation for cold adaptation traits appears to be additive (discussed below). If Q_{st} equals F_{st} (i.e., for neutral genetic markers), then natural selection is probably having little influence on population differentiation for the quantitative trait (Merilä and Crnokrak 2001). If Q_{st} exceeds F_{st}, then selection for alternative phenotypes is probably contributing to population differentiation. If Q_{st} is less than F_{st}, then stabilizing selection for the same phenotype in different populations is probably limiting population differentiation (i.e., below what would result from genetic drift alone).

Q_{st} values for cold adaptation traits vary widely in forest trees (Table 1). Nonetheless, most (16 of 21) of the estimates are greater than the corresponding estimate of F_{st}, suggesting that natural selection is favoring different cold adaptation phenotypes in different populations. The geographic scale across which populations are sampled has a substantial effect on the amount of variation among populations but should not influence estimates of within-population variance. Thus, range-wide samples should produce higher Q_{st} estimates than regional experiments. For example, the very large Q_{st} estimate for *Picea mariana* (0.91) is from a range-wide experiment (Table 1). In nearly all cases (7 of 9), Q_{st} is higher for bud set than for bud flush.

Genetic clines in cold adaptation: further evidence for natural selection

Population differences in cold adaptation are not random; they are strongly associated with climatic and geographic gradients. Consistent relationships are found between cold adaptation traits and a number of climatic and geographic variables. These trends, which have been reviewed elsewhere (Morgenstern 1996; Howe et al. 1995, 2000; Aitken and Hannerz 2001), provide compelling indirect evidence that these traits are under strong natural selection.

In general, trees from colder regions (i.e., more northern, higher elevation, and more continental locations) flush earlier and are more susceptible to spring frosts in common garden experiments (Morgenstern 1996; Howe et al. 2000; Aitken and Hannerz 2001). Early bud flush could result from either a low chilling requirement, low heat sum requirement, or a combination of the two. In Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*), for example, trees from higher elevations and more continental locations (i.e., farther from the

Table 1. Q_{st} estimates for cold adaptation traits and F_{st} estimates (i.e., G_{st}) for allozyme markers.

Species	Mean Q_{st}			F_{st}	References	
	Bud set or growth cessation	Bud flush or growth initiation	Cold injury		Q_{st}	F_{st}
Larix occidentalis	0.50[a]	0.08	—	0.086	Rehfeldt 1992*a*	Fins and Seeb 1986
Picea glauca	0.25[a]	0.05[a]	—	0.014	Jaramillo-Correa et al. 2001	Jaramillo-Correa et al. 2001
	0.15[a]	1.00[a]	—	—	Li et al. 1993	
Picea mariana	0.91[a]	0.52[a]	—	0.059	Morgenstern 1969	Yeh et al. 1986
Picea sitchensis	0.08	0.06	—	0.079	Campbell et al. 1989	Yeh and El-Kassaby 1980
Pinus ponderosa	0.05	0.02	—	0.122	Rehfeldt 1992*b*	O'Malley et al. 1979
Pinus sylvestris	0.82[a]	—	—	0.02	Hurme 1999	Karhu et al. 1996
Pseudotsuga menziesii var. *glauca*	0.20[a]	0.12[a]	0.47[ab]	0.043	Rehfeldt 1978	Li and Adams 1989
Pseudotsuga menziesii var. *menziesii*	0.13[a]	0.11[a]	—	0.071	Campbell 1986	Li and Adams 1989
Populus balsamifera[c]	0.09[a]	0.13[a]	—	0.014	Riemenschneider et al. 1992; Farmer 1993	Farmer et al. 1988
Populus tremuloides[c]	—	0.14[a]	—	0.068	Thomas et al. 1997	Hyun et al. 1987

[a]Because Q_{st} is much greater than F_{st}, this provides evidence for differential natural selection among populations (Merilä and Crnokrak 2001; McKay and Latta 2002).
[b]Fall cold hardiness.
[c]Q_{st} may be biased downward for these species because within-population variance was estimated from variance among clones, which may include nonadditive genetic effects and (or) nongenetic clonal effects.

ocean) have lower chilling requirements to break dormancy (Campbell and Sugano 1979). Exceptions to these trends are probably due to the complex interactions between chilling requirements, heat sum requirements, and test environments (Kriebel and Wang 1962; Campbell and Sugano 1979). The relationship between geographic origin and spring frost hardiness may be weaker than it is for bud set. This is because there is less genetic variation for bud flush than for bud set (Aitken and Hannerz 2001). Cannell and Sheppard (1982), for example, observed earlier dehardening in southern provenances of *Picea sitchensis*, but did not observe variation in the timing of bud flush.

Genotypes from colder regions with shorter growing seasons also tend to stop growing and set bud earlier in the fall (Kuser and Ching 1980; Skrøppa and Magnussen 1993; Morgenstern 1996; Aitken and Hannerz 2001). Because early growth cessation is associated with increased fall frost hardiness, these genotypes also tend to be shorter and more frost hardy (Aitken and Hannerz 2001).

In recent years, genetic clines have been studied in relation to climatic variables, rather than geographic surrogates for climate. In these studies, climatic variables are usually derived from climatic models (e.g., Rehfeldt et al. 1999). Frost hardiness, bud set, and (to a lesser extent) bud flush are associated with the temperature regime at the population's origin, including the number of frost-free days, frost-free period, and mean or minimum temperatures either throughout the year, or in specific months (Campbell 1974; Benowicz et al. 2001; J.B. St. Clair, USDA Forest Service, Corvallis, Oreg., pers. commun.). Genotypes from colder environments, for example, have more conservative growth patterns and greater cold hardiness in situ than those from milder environments, but lower chilling requirements can result in early bud flush of cold-adapted trees when planted in milder environments. In some cases, gradients in summer precipitation also reveal clines in growth cessation that reduce susceptibility to injury from both late summer droughts and low temperatures in the fall (*Pseudotsuga menziesii*; White 1987; Joly et al. 1989).

Sympatric species can vary considerably in their degree of local adaptation. For example, *Pseudotsuga menziesii* and *Pinus contorta* show a high degree of local adaptation, whereas other species such as *Pinus monticola* show almost no geographic differentiation (i.e., based on multivariate analyses of traits such as height growth, timing of bud set, bud flush, and cold injury). Genetic differentiation can occur among populations separated by more than 100–200 m for *Pseudotsuga menziesii* var. *glauca* and *Pinus contorta*; 500 m for *Larix occidentalis*; and 600 m for *Thuja plicata* (Rehfeldt 1979*a*, 1979*c*, 1983*a*, 1994, 1995). No elevational differentiation was observed for *Pinus monticola*, however (Rehfeldt 1979*b*; Rehfeldt et al. 1984). The very late growth initiation and rapid elongation of *Pinus monticola* may facilitate its generalist adaptational approach (I. Chuine and S.N. Aitken, unpublished data).

We conclude that populations are often well differentiated for cold adaptation traits but usually weakly differentiated for molecular genetic markers such as allozymes, i.e., estimates of Q_{st} for cold adaptation traits are usually larger than estimates of F_{st} (Table 1). Furthermore, the ubiquity of genetic clines that are associated with climatic gradients provides convincing evidence that natural selection is responsible for these patterns of differentiation. These conclusions have important implications for gene resource management because they suggest that population differentiation may be severely underestimated based on neutral genetic markers.

Cold adaptation of reproductive tissues

One area of genecology that is critically important but poorly understood is population variation in cold adaptation

of reproductive tissues (e.g., Hannerz et al. 2001). In many tree species, reproductive bud flush precedes vegetative bud flush, and reproductive structures are generally quite vulnerable to cold injury. In *Rhododendron*, for example, flower buds are the most susceptible overwintering organs (Väinölä 2000), and reproductive organs are generally more susceptible than vegetative organs during the growing season (Bigras et al. 2001). Selection for delayed reproductive events in the spring may play a role in restricting species distributions by limiting the range of environments over which seed can be fully matured in the available growing season (I. Chuine and S.N. Aitken, unpublished data). To determine the lifetime fitness of genotypes in a changing climate, the effects of climate on reproductive phenology must be better understood.

Substantial within-population variation for cold adaptation

Although natural selection has led to population differentiation, substantial genetic variation remains within populations, often accounting for more than half of the total genetic variation ($1 - Q_{st}$; Table 1). This may reflect heterogeneity of natural selection, both spatially and temporally, or high levels of gene flow (Campbell 1979; Hamrick et al. 1992; Hamrick and Nason 2000). Most mortality due to cold injury occurs in young seedlings, although there are examples of extreme cold events resulting in mortality of large trees (e.g., *Tsuga heterophylla*, *Alnus rubra*; Duffield 1956). If seedlings become established during a period in which severe frosts are absent, a cohort may avoid selection for cold adaptation. In addition, the severity of frosts varies considerably with microtopography and exposure. Within stands, seedlings may be exposed to different selection regimes if they are in a topographic depression, on a raised microsite, or protected by overstory vegetation. Because interprovenance seed transfer is used with caution in many tree improvement programs, the large amount of within-population variation is available for improving cold adaptation via selection and breeding.

Quantitative genetics

Genetic control of cold adaptation

Phenological traits are under moderate to strong genetic control (Table 2). On average, well over 50% of the variation in common garden experiments can be attributed to genetic causes, which is generally much higher than for size-related traits (Zobel and Talbert 1984). In a number of experiments, the heritability of bud phenology was higher than that of any other measured trait (Riemenschneider et al. 1992; Bradshaw and Stettler 1995). In general, bud flush is under slightly stronger genetic control than is bud set. This may reflect the numerous environmental cues that influence the timing of growth cessation and bud set, particularly in young seedlings. Although bud set is readily influenced by photoperiod, temperature, soil moisture, nutrition, and light quality, bud flush is mostly influenced by temperature (at least under normal field conditions). Despite these trends, genetic control of cold adaptation is complex, varying among genotypes, environments, and developmental stages.

Bud set exhibits more genetic variation than does bud flush, both among populations and individuals (Aitken and Hannerz 2001; Howe et al. 2000). For four conifer species in seven experiments, the range between the earliest and latest bud-flushing population averaged slightly less than 4 d, whereas the range in bud set averaged over 20 d (Aitken and Hannerz 2001). For bud phenology, the majority of genetic variation appears to be additive, at least for bud flush and bud set in *Picea abies* (Eriksson et al. 1978; Ekberg et al. 1991). Furthermore, broad sense and narrow sense heritabilities for these traits are typically similar (Table 2).

The genetics of cold injury have been studied using artificial freeze tests and by measuring damage from natural freeze events in the field (Aitken and Adams 1996, 1997; O'Neill et al. 2000, 2001; Howe et al. 2000). Heritabilities for cold injury vary with the season of sampling; spring cold injury is under strong genetic control, whereas the genetic control of fall cold hardiness is both moderate and tissue specific. Genetic variation in midwinter hardiness has been documented (e.g., *Betula pendula*, Li et al. 2003*b*; *Pinus sylvestris* and *P. contorta*, Nilsson 2001), but differences are low or nonsignificant in many species (e.g., *Pseudotsuga menziesii*, Aitken and Adams 1996; *Betula papyrifera*, Benowicz et al. 2001; *Quercus petraea*, Deans and Harvey 1996). Genetic variation in fall and spring cold hardiness largely results from differences in the timing of acclimation and deacclimation.

Genotype × environment and genotype × developmental-stage interactions

For genotypes of Douglas-fir, the relative timing of bud flush is consistent between seedlings and saplings. This is evidenced by high genetic correlations between different trees from the same families at different ages (O'Neill et al. 2000). In an F_2 family of hybrid poplar, genetic correlations for bud set were high ($r_g = 0.81$) between field sites in highly contrasting environments in Minnesota and Oregon, but were considerably lower (average 0.56) between bud set in the field and bud set under short days (8 h) in the greenhouse, and even lower (average 0.38) between bud set in the field and bud set under a natural fall photoperiod in the greenhouse (Howe et al. 2000).

In Douglas-fir, strong genetic correlations ($r_a = 0.64$–1.0) have also been observed among sites and years for cold hardiness measured in the early fall and spring (Aitken and Adams 1996, 1997). For cold hardiness measured in late fall and winter, however, genotype × site and genotype × tissue interactions were substantial. Therefore, although the expression of cold adaptation traits is relatively robust, there is at least the potential for substantial genotype × environment interactions. Traditional quantitative genetic approaches cannot elucidate the genetic mechanisms triggering such interactions, but studies of individual quantitative trait loci (QTL) have the potential to do so.

Genetic correlations among traits

Spring cold injury is strongly genetically correlated with the timing of bud flush in most species, whereas the relationship between timing of bud set and fall cold hardiness is variable. In Douglas-fir, the timing of bud set in 1-year-old seedlings was genetically correlated with fall cold hardiness, but in saplings (which exhibit seasonally determinate growth) bud set occurred much earlier in the summer and was more strongly correlated with bud flush, reflecting the

Table 2. Narrow-sense (h_i^2) or broad-sense (H_i^2) individual-tree heritabilities for the timing of bud flush or bud set; lignification; second flushing; fall, winter, or spring frost hardiness from artificial freeze tests; and fall or spring frost damage from natural, outdoor frosts.

Trait	Species	Heritability median (range)[a]	References
Bud flush			
	Picea abies	0.82 (0.75–0.89)	Hannerz et al. 1999*b*
	Picea glauca	0.42 (0.31–0.70)	Nienstaedt and King 1970; Wilkinson 1977; Nienstaedt 1985
	Pseudotsuga menziesii var. *glauca*	0.52	Rehfeldt 1983*b*
	Pseudotsuga menziesii var. *menziesii*	0.87 (0.45–1.0)	Aitken and Adams 1995*a*, 1995*b*; Christophe and Birot 1979; El-Kassaby and Park 1993; Li and Adams 1993, 1994
	Populus balsamifera	0.25 (0.21–0.47)[b]	Farmer 1993
	Populus trichocarpa × *P. deltoides*	0.80[b]	Howe et al. 2000
	Robinia pseudoacacia	0.62 (0.55–0.83)	Mebrahtu and Hanover 1989
Bud set			
	Pseudotsuga menziesii var. *glauca*	0.25	Rehfeldt 1983*b*
	Pseudotsuga menziesii var. *menziesii*	0.70 (0.16–0.81)	Aitken and Adams 1995*a*, 1995*b*; Li and Adams 1993
	Populus balsamifera	0.65[b]	Riemenschneider et al. 1992
	Populus trichocarpa	0.69 (0.62–0.76)[b]	Weber et al. 1985; Riemenschneider et al. 1994
	Populus trichocarpa × *P. deltoides*	0.53 (0.48–0.72)[b]	Howe et al. 2000
Lignification			
	Picea abies	0.34 (0.21–0.48)	Hannerz et al. 1999*b*
Second flushing			
	Picea abies	0.21 (0.19–0.24)	Hannerz et al. 1999*b*[c]
	Pseudotsuga menziesii var. *glauca*	0.32	Rehfeldt 1983*b*
	Pseudotsuga menziesii var. *menziesii*	0.45 (0.25–0.66)	Aitken and Adams 1995*a*
Fall hardiness			
	Pseudotsuga menziesii var. *menziesii*	0.19 (0.16–0.28)	Aitken and Adams 1995*a*, 1995*b*, 1996; Aitken et al. 1996
Winter hardiness			
	Pseudotsuga menziesii var. *menziesii*	0.11 (0.0–0.35)	Aitken and Adams 1996
Spring hardiness			
	Pseudotsuga menziesii var. *menziesii*	0.77 (0.56–0.78)	Aitken and Adams 1995*a*, 1995*b*, 1997
Fall frost damage			
	Picea abies	0.27	Hannerz et al. 1999*b*
	Populus trichocarpa × *P. deltoides*	0.27[b]	Howe et al. 2000
Spring frost damage			
	Picea glauca	0.12 (0.12–0.13)	Nienstaedt 1985
	Pseudotsuga menziesii var. *menziesii*	0.56	Aitken and Adams 1997

Note: Conifer values are modified from Aitken and Hannerz (2001).

[a]For entries derived from multiple publications, the median and ranges are mostly based on a single value from each of the cited references (i.e., mean heritabilities were calculated from all values reported in the publication). However, in cases where the traits differed substantially (e.g., traits measured on trees of very different ages), the median and ranges may include multiple values from a single publication. For entries derived from a single publication, the median and ranges are based on all heritabilities reported. Unless noted otherwise, heritability values represent h_i^2 derived from analyses of progeny tests.

[b]Values represent H_i^2 derived from analyses of clonal tests.

[c]The trait measured was "free growth", which was mostly due to second flushing.

developmental time necessary for elongation of the predetermined shoot (O'Neill et al. 2000; Li and Adams 1993). In *Populus*, which exhibits seasonally indeterminate growth, fall frost damage was positively correlated (r_g = 0.72) with the timing of bud set in the environment in which the clones were growing (Minnesota), as well with the timing of bud set of the same clones growing in Oregon (r_g = 0.57) (Howe et al. 2000). Significantly, survival through the winter was negatively related to both frost damage (r_g = –0.70) and the timing of bud set (r_g = –0.30).

Cold injury in the spring and fall are either unrelated to one another or have weak negative genetic correlations in both seedlings and saplings of Douglas-fir (O'Neill et al. 2000, 2001). Fall cold acclimation and dormancy induction are temporally correlated in many species (Sakai and Larcher 1987; Hänninen et al. 2001), but phenotypic studies alone cannot determine to what extent these are functions of the same sets of genes or different genes responding to the same environmental cues.

Tradeoffs between growth and cold hardiness

At broad geographic scales, strong tradeoffs often exist between the amount of seasonal growth and cold hardiness, largely because of a positive genetic correlation between the duration of primary growth and the total amount of elongation accomplished (Table 3). Genetic differences in growing season length are determined more by the timing of growth cessation than by the timing of growth initiation (Aitken and

Hannerz 2001). If trees grow too late into the fall, they will be susceptible to frost injury and mortality, but if primary growth stops too early in the season, trees will be less able to compete for light and other resources (Aitken and Hannerz 2001). Patterns of geographic variation in drought hardiness often resemble those of cold hardiness. In Douglas-fir, similar negative correlations among population means have been observed between growth rate and drought hardiness traits, with populations from drier environments having a genetically determined, shorter growing season, earlier bud flush and bud set, and higher root/shoot ratios (White 1987; Joly et al. 1989; Kaya et al. 1994).

The tradeoffs between growth and cold hardiness are usually strong at the population level, but weaker and less consistent within populations (Aitken and Hannerz 2001). For example, the negative genetic correlations between growth and cold hardiness are weaker and more variable within than among populations (Table 3). Thus, the impacts of selection for growth on cold adaptation cannot be predicted, but must be estimated empirically for each species and even for populations within species. Furthermore, it is unclear whether these correlations result from pleiotropy or linkage disequilibrium (LD). The unpredictable within-population relationships between growth and cold hardiness suggest the latter is more likely. If the correlations result from pleiotropy, it may be impossible to improve both traits simultaneously; but if they result from LD, then it should be possible to make simultaneous breeding progress in both traits.

Cold adaptation and gene resource management

Knowledge about cold adaptation is key to conserving and managing the forest genetic resources of temperate and boreal regions (Aitken 2000). Gene conservation efforts are usually designed to protect representative, genetically distinct populations; and in these regions, populations are more likely to vary for cold adaptation than for any other trait. In contrast, little genetic differentiation is seen over wide geographic areas for selectively neutral genetic markers (Hamrick et al. 1992). The identification of populations for conservation (Aitken 2000) and the development of seed transfer guidelines and delineation of breeding zones (Campbell 1974) require information on quantitative traits, including growth and traits directly related to adaptation to cold and drought.

Breeding programs for native tree species typically exclude cold hardiness as an explicit goal, although the risk of maladaptation is often a concern (e.g., Rehfeldt 1992*a*, 1992*b*). Maladaptation may result if breeding zones are too large, or if the traits under selection (e.g., growth rate) have adverse genetic correlations with cold hardiness (Aitken and Hannerz 2001). More commonly, increased cold hardiness is a breeding objective for exotic species such as coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) in France (Heois 1994), Sitka spruce (*Picea sitchensis*) in Scotland (Cannell et al. 1985*a*, 1985*b*), and *Eucalyptus* throughout the world (Byrne et al. 1997). Cold adaptation should also be a concern for programs that focus on producing genetically modified trees, because the genotypes that are genetically modified must also be adapted to the climate of the intended planting area.

Quantitative trait loci: pedigree studies

The inheritance of quantitative traits can be studied by measuring the co-segregation of phenotypes and genetic markers within families (pedigrees) (Sewell and Neale 2000). Using this approach, one may be able to estimate the number, genomic positions, magnitudes of effects, gene actions, heritabilities, and interactions (e.g., pleiotropic and environmental) of the genetic factors, or quantitative trait loci (QTL), that are involved (Zeng et al. 1999). In contrast, it may be difficult or impossible to identify the individual genes that control quantitative traits, or to find genetic markers for broad use in marker-assisted selection across different pedigrees or populations. Nonetheless, QTL analyses are valuable for shedding light on the genetic architecture of quantitative traits, for better understanding the physiology of traits such as cold adaptation, and for prioritizing candidate genes for further study (Zeng et al. 1999; Frewen et al. 2000; Jermstad et al. 2003).

Cold adaptation traits are mostly controlled by multiple quantitative trait loci with small effects

Cold adaptation QTL have been detected in both conifers and angiosperms using approaches similar to those described by Sewell and Neale (2000). QTL for fall cold hardiness, spring cold hardiness, vegetative bud set, and vegetative bud flush have all been detected in both pure species and hybrid pedigrees (Table 4). These studies indicate that cold adaptation traits are mostly controlled by multiple QTL with relatively small effects; for example, as many as 33 QTL were detected for spring bud flush in Douglas-fir (Jermstad et al. 2001*a*). The percentages of phenotypic variance explained (PVE) are usually less than 10%. Because these PVE values reflect the amounts of phenotypic, not genetic, variation explained, the proportions of genetic variation explained by the QTL will be larger than the PVE, and these differences will be most pronounced for low heritability traits. In contrast, we should also consider that the PVEs listed in Table 4 are overestimates. QTL effects are biased upwards whenever the locations and phenotypic effects of QTL are estimated from a single data set (Göring et al. 2001), and overestimation is exacerbated by small sample sizes (Beavis 1998).

In general, more QTL with smaller effects were detected in Douglas-fir than in Scots pine, *Eucalyptus*, *Populus*, and *Salix* (Table 4). Although some of these differences may reflect real biological differences, other factors may be involved, including variation in study design, statistical approaches, and the number of traits examined (i.e., in Douglas-fir, cold hardiness was measured separately in needles, buds, and stems; Jermstad et al. 2001*b*).

The Scots pine study was specifically undertaken to map QTL responsible for adaptational differences between northern (66°35′N) and southern (60°37′N) populations in Finland (Hurme et al. 2000). The authors detected four QTL for bud set and seven for frost hardiness and concluded that alternative alleles of "rather large" effect have been selected in these populations. Experiments in *Pseudotsuga menziesii*, *Eucalyptus*, and *Populus* were also initiated by choosing grandparents from divergent populations to maximize segregating variation in the F_2 generation. In Douglas-fir, the grandparents were chosen from two breeding zones that dif-

Table 3. Among-population and within-population correlation coefficients between cold adaptation traits and growth (e.g., total height, biomass, or height increment).

Trait and species[b]	Age-class (years)	Correlation (r)[a] Among populations	Within populations	Reference
Bud set vs. growth				
Picea abies	1–3	—	0.29[c]	Pulkkinen 1993
Picea abies	1–3	0.78	—	Hannerz and Westin 2000
Picea glauca	1–3	—	0.57[d]	Li et al. 1993
Picea glauca × *P. engelmannii*[f]	1–3	0.59	—	Roche 1969
Picea sitchensis	1–3	0.77	—	Falkenhagen 1977
Pseudotsuga menziesii var. *glauca*	1–3	0.86	—	Rehfeldt 1979*a*
Pseudotsuga menziesii var. *glauca*	4–20	—	0.64[d]	Rehfeldt 1983*b*
Pseudotsuga menziesii var. *menziesii*	1–3	—	0.77[d]	Li and Adams 1993
Pseudotsuga menziesii var. *menziesii*	4–20	—	0.49[d]	Li and Adams 1993
Pseudotsuga menziesii var. *menziesii*	1–3	0.79[d]	0.71[d]	Campbell 1986
Populus balsamifera	1–3	—	0.63[c,e]	Riemenschneider et al. 1992
Populus trichocarpa	1–3	—	0.19[c,e]	Riemenschneider et al. 1994
Growth cessation vs. growth				
Picea mariana	1–3	0.83	—	Morgenstern 1978
Pinus monticola	1–3	0.71	—	Rehfeldt et al. 1984
Pinus ponderosa	4–20	—	0.50[d]	Rehfeldt 1992*b*
Larix occidentalis	4–20	—	0.62[d]	Rehfeldt 1992*a*
Fall cold injury vs. growth				
Picea abies	4–20	—	–0.22	Skrøppa 1991
Picea abies	1–3	0.51–0.68	—	Hannerz and Westin 2000
Pinus contorta	1–3	0.82	—	Rehfeldt 1987
Pinus contorta	4–20	0.81	—	Rehfeldt 1987
Pinus monticola	1–3	0.51	—	Rehfeldt et al. 1984
Pseudotsuga menziesii var. *glauca*	1–3	0.70	—	Rehfeldt 1979*a*
Pseudotsuga menziesii var. *menziesii*	4–20	—	0.19[d]	Aitken et al. 1996
Tsuga heterophylla	4–20	0.91	–0.27	Hannerz et al. 1999*a*
Bud flush vs. growth				
Picea glauca[f,g]	4–20	—	0.32	O'Reilly and Parker 1982
Picea glauca	4–20	—	0.12	Nienstaedt 1985
Picea glauca × *P. engelmannii*[f]	1–3	–0.18	—	Roche 1969
Picea mariana[g]	4–20	—	–0.57	O'Reilly and Parker 1982
Picea sitchensis	1–3	–0.04	—	Falkenhagen 1977
Pseudotsuga menziesii var. *glauca*	1–3	–0.27	—	Rehfeldt 1979*a*
Pseudotsuga menziesii var. *menziesii*	1–3	–0.31[d]	–0.41[d]	Campbell 1986
Pseudotsuga menziesii var. *menziesii*	1–3	—	0.20[d]	Li and Adams 1993
Pseudotsuga menziesii var. *menziesii*	4–20	—	0.51[d]	Li and Adams 1993
Tsuga heterophylla	4–20	–0.72	0.28	Hannerz et al. 1999*a*
Robinia pseudoacacia	1–3	—	0.97[d]	Mebrahtu and Hanover 1989
Growth initiation vs. growth				
Larix occidentalis	4–20	—	–0.42[d]	Rehfeldt 1992*a*
Picea mariana	1–3	0.91	—	Morgenstern 1978
Pinus monticola	1–3	–0.66	—	Rehfeldt et al. 1984
Pinus ponderosa	4–20	—	–0.23[d]	Rehfeldt 1992*b*
Spring cold injury vs. growth				
Picea glauca	4–20	—	0.32	Nienstaedt 1985
Pinus contorta	4–20	–0.43	—	Rehfeldt 1987
Pseudotsuga menziesii var. *glauca*	1–3	0.79	—	Rehfeldt 1979*a*
Tsuga heterophylla	4–20	0.80	0.43	Hannerz et al. 1999*a*
Growth duration vs. growth				
Larix occidentalis	4–20	—	0.70[d]	Rehfeldt 1992*a*
Picea glauca	1–3	—	0.63[d]	Li et al. 1993
Pinus monticola	1–3	0.78	—	Rehfeldt et al. 1984
Pinus ponderosa	4–20	—	0.61[d]	Rehfeldt 1992*b*

Table 3 (*concluded*).

Trait and species[b]	Age-class (years)	Correlation (r)[a] Among populations	Within populations	Reference
Pseudotsuga menziesii var. *menziesii*	1–3	—	0.64[d]	Li and Adams 1993
Pseudotsuga menziesii var. *menziesii*	4–20	—	–0.25[d]	Li and Adams 1993
Growth rate vs. growth				
Larix occidentalis	4–20	—	0.62[d]	Rehfeldt 1992*a*
Pinus monticola	1–3	0.85	—	Rehfeldt et al. 1984
Pinus ponderosa	4–20	—	0.79[d]	Rehfeldt 1992*b*

[a]For phenological traits, positive correlations indicate that later bud flush or bud set is associated with greater growth. Where necessary (e.g., for percent bud set), the signs of these correlations were reversed from the original values. Data are phenotypic correlations based on family or population means unless otherwise designated.
[b]Growth was measured as total height except for where noted.
[c]Rank correlation coefficient.
[d]Genetic correlation based on additive genetic variances and covariance from progeny test analysis.
[e]Genetic correlation based on total genetic variances and covariance from clonal test analysis.
[f]Growth was measured as dry mass.
[g]Growth was measured as height increment.

fer in the timing of bud flush (Jermstad et al. 2001*a*). In poplar, a three-generation inbred-like pedigree was derived from an interspecific cross between a black cottonwood tree from Washington and an eastern cottonwood tree from Texas (Frewen et al. 2000). Therefore, the QTL effects detected may include differences between species and between latitudes within species (discussed below). In *Eucalyptus*, QTL analyses were based on a three-generation pedigree derived from four grandparents from four provenances that differ in frost hardiness (Byrne et al. 1997). In contrast with the other studies, the Scots pine experiment used an "open-pollinated backcross" design, rather than a three-generation full-sib design, and fewer progeny, both of which tend to result in the detection of fewer QTL with larger effects.

It is difficult to tie these differences in QTL number and magnitude of effects to any particular factor. Despite these differences, the results presented in Table 4 support an oligogenic or polygenic model of inheritance for traits conferring adaptation to cold, presumably involving tens to hundreds of loci. Similar conclusions have been drawn for other traits in forest trees (Sewell and Neale 2000). Results from QTL experiments in a wide range of organisms are inconsistent: some suggest the presence of a few QTL with large effects, but others suggest the presence of many QTL with much smaller effects (reviewed in Sewell and Neale 2000; Barton and Keightley 2002; Table 4). There are two main explanations for these discrepancies. First, QTL with large effects may actually exist. In intraspecific mapping experiments, alleles with large effects could be present because of the constant influx of new mutations into the population (Barton and Keightley 2002). In interspecific mapping experiments, large effects may be found by uncovering divergent sets of alleles that are fixed in the two species, alleles that may not be detected through intraspecific analyses of the two parental species. For a host of reasons (including the methodological reasons discussed above), QTL experiments tend to underestimate the number of QTL and overestimate their effects (Barton and Keightley 2002, Box 1). In a typical experiment, for example, no more than about 12 QTL are likely to be detected (Hyne and Kearsey 1995; reviewed in Barton and Keightley 2002). Therefore, our preferred working hypothesis is that cold adaptation in forest trees is influenced by many QTL with small effects, particularly for the variation that is observed within species. Whether the QTL that vary among species are qualitatively different from those that differ among or within populations remains an open and important question. To answer this question, it would be best to use a single experiment to compare the results of within-population QTL analyses with those that focus on differences among populations and species. Because this would be difficult to do using pedigree analyses, approaches that associate precise genetic markers with adaptive phenotypes in large populations of unrelated individuals (i.e., association studies) are more likely to shed light on these important questions (see Quantitative trait loci: Association studies).

Correlations among quantitative trait loci: evidence for pleiotropy

QTL analyses may be valuable for understanding the biological basis of genetic correlations among traits, i.e., pinpointing pleiotropy, tight linkage, or other causes of LD as the basis of trait correlations. As noted above, there are consistently strong genetic correlations between the timing of spring bud flush and spring cold hardiness in a wide range of species. Therefore, it should be possible to identify those QTL that have the greatest contribution to this covariance. In Douglas-fir, three QTL for spring cold hardiness mapped to the same locations as QTL for spring bud flush (Jermstad et al. 2001*b*). Other strong genetic correlations are found between the timing of bud set and fall cold hardiness in some species, particularly at the seedling stage. Although there was no obvious relationship between the QTL for bud set and cold hardiness in Scots pine (Hurme et al. 2000), one of the two cold hardiness QTL detected in *Populus* mapped to the same location as one of the bud set QTL (i.e., the timing of short-day-induced bud set in the greenhouse) (Chen et al. 2002; G.T. Howe, T.H.H. Chen, and H.D. Bradshaw, unpublished data).

Allele effects for quantitative trait loci are difficult to predict from the phenotype of the parent

The direction, or sign, of the effect of a QTL allele is difficult to predict from the phenotype of the parent. The

Table 4. Cold adaptation quantitative trait loci (QTL) in forest trees.

Species or hybrid	Trait	*N*	No. of QTL	PVE (%)[a]	Unpredicted allele effects (%)[b]	References
Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*)	Fall cold hardiness[c]	≤184	11	2.0–6.8	—	Jermstad et al. 2001*b*
	Spring cold hardiness[c]	≤184	15	1.4–9.8	28	Jermstad et al. 2001*b*
	Spring bud flush[d]	≤190	33	1.2–11.5	35	Jermstad et al. 2001*a*
Scots pine (*Pinus sylvestris*)	Fall cold hardiness	53[e]	7	0.01–12.2	—	Hurme et al. 2000
	Fall bud set	≤92[e]	4	3.5–14.5	—	Hurme et al. 2000
Scots pine (*Pinus sylvestris*)	Fall cold hardiness	94	2	11.3–22.7	—	Lerceteau et al. 2000
Populus (*P. trichocarpa* × *P. deltoides*)	Fall frost damage	286	2	9.5–48.8[f]	0[g]	Chen et al. 2002; data not included
	Fall bud set[h]	346	6	5.5–18.0	17	Frewen et al. 2000; Chen et al. 2002
	Spring bud flush[h]	337	6	7.2–18.5	30	Frewen et al. 2000; Chen et al. 2002
	Short-day-induced bud set	334	4–5	5.9–28.8	20	Chen et al. 2002; data not included
	Chilling response	100	1	18.2	—	Chen et al. 2002; data not included
	Winter survival	326	0	—	—	Chen et al. 2002; data not included
Eucalyptus (*E. nitens*)	Frost tolerance	118	2	7.7–10.8	—	Byrne et al. 1997
Willow hybrids (*Salix viminalis* × [*S. viminalis* × *S. schwerinii*])	Bud flush (field)	92	4	13–15[i]	—	Tsarouhas et al. 2003; V. Tsarouhas, pers. comm.
	Bud flush (indoors)	92	2–5	6–16	—	Tsarouhas et al. 2003

[a]PVE is the percentage of total phenotypic variance in the progeny that was explained by the QTL. The listed range includes all PVE estimates reported, including multiple estimates for the same QTL that were derived using alternative statistical approaches.

[b]Values indicate the percentages of additive QTL effects that do not match predictions based on the grandparental phenotypes from which the alleles were derived. For example, if an early-flushing grandparent transmits an allele that contributes to late-flushing progeny, then this would be counted as a mismatch. A dash indicates that the grandparental phenotype is not known and cannot be reasonably inferred.

[c]Combined results for separate QTL analyses of needles, buds, and stems.

[d]Combined results for separate QTL analyses of terminal and lateral bud flush in Oregon and Washington in years 1995–1998.

[e]Different sets and numbers of individuals were used for mapping QTL via selective genotyping and for estimating QTL effects. The value presented is the number of observations used in ANOVA (Table 2, Hurme et al. 2000).

[f]A frost hardiness QTL that displayed overdominant gene action was the one that had a PVE value of 48.3%.

[g]Although the additive effects of the two frost hardiness QTL were as expected, one of the QTL displayed overdominant gene action. Trees that were heterozygous for this QTL allele had enhanced fall cold hardiness.

[h]Values differ from those reported in Frewen et al. (2000), because subsequent analyses included bud set measured in Minnesota and additional codominant markers (see Chen et al. 2002).

[i]V. Tsarouhas, personal communication.

high-scoring parent in a pedigree often passes on alleles that contribute to a low-scoring phenotype. This occurs, for example, when a cold-susceptible parent contributes QTL alleles that enhance cold hardiness in the progeny. These "unpredicted" QTL effects are common and have important implications for tree breeding. For the species and traits reported in Table 4, for example, about a third of the estimated QTL effects did not coincide with the phenotype of the parent (i.e., the sign of the QTL effect was negative; Jermstad et al. 2001*b*). Northern and southern populations of *Populus*, for example, are strongly differentiated for photoperiodic responses and the timing of bud set; southern genotypes set bud later than do northern genotypes in common garden experiments (Howe et al. 1995). Despite these differences, QTL analyses demonstrated that a southern tree from Texas contributed alleles that advanced fall bud set by about 12.6 d compared with the alleles from the Washington parent (Frewen et al. 2000). These results can be explained by the high levels of within-population variation for cold adaptation and demonstrate that divergent populations are not necessarily fixed for divergent alleles at all loci. Furthermore, these results suggest that wide crossing may be advantageous in advanced generation breeding programs, even when a breeder desires to maintain or improve adaptability; i.e., transgressive segregation is likely in the F_2 generation of wide-cross pedigrees.

Quantitative trait loci × environment effects are common

Genotype by environment (G × E) interactions are common in forest trees. Within Douglas-fir breeding zones, for example, genetic correlations among test sites are only about 0.7 for height growth (Johnson 1997). Therefore, it is not surprising that QTL × environment (QTL × E) interactions are similarly common. Two QTL × treatment interactions were detected for the timing of growth initiation in Douglas-

fir, whereas several were detected for the timing of growth cessation (Jermstad et al. 2003). Despite these interactions, it was possible to detect some of the same QTL in multiple environments. In *Populus* hybrids, we found evidence for QTL × E interactions for bud set between field tests in Minnesota and Oregon, and between field tests and controlled environments (Chen et al. 2002; G.T. Howe, T.H.H. Chen, and H.D. Bradshaw, unpublished data). Despite these interactions, two of the four bud set QTL that we detected in Minnesota were also detected in Oregon. Given these QTL × E interactions, the challenge for ecological geneticists and tree breeders is to identify those QTL that are reliably detected across environments and to explain the QTL interactions whenever possible (i.e., by better characterizing the field environments and physiological responses). These environments should include both complex field environments and carefully controlled environments (e.g., long vs. short days; warm vs. cold temperatures) that can be used to dissect the genetic control of complex physiological traits (Howe et al. 2000; Jermstad et al. 2003). There is ample evidence that QTL effects for other traits vary with the environment, developmental stage, and genetic background in forest trees, underscoring the need for QTL verification in independent experiments (Sewell and Neale 2000).

Limitations of pedigree studies

The substantial LD that exists within pedigrees is both a blessing and a curse. First, the large LD that exists within typical QTL mapping populations means that even markers located far from QTL (e.g., 10–20 cM) co-segregate with QTL alleles. This is what makes it possible to detect marker–QTL associations using relatively few progeny and genetic markers. On the other hand, this is also what makes it so difficult to identify the underlying genes or use the linked markers for marker-assisted selection in more than one family. In typical QTL experiments, it is difficult to get much closer than a few centimorgans to the QTL of interest, which could correspond to hundreds or thousands of kilobases and a very large number of genes. LD is expected to be low within natural populations of forest trees because of typically high levels of outcrossing, high dispersal distances for pollen, and large effective population sizes. This means that the genetic markers that are associated with desirable QTL alleles in one family will probably not be helpful in another, although this may not apply to hybrids between species or provenances.

Quantitative trait loci: association studies

Genetic association studies involve searching for statistical associations between phenotypes and marker alleles in populations of unrelated individuals. In contrast with pedigree analyses, which involve detecting co-inheritance (i.e., linkage) between QTL and genetic markers over a few generations, association studies involve detecting co-inheritance that has persisted over many generations (see Box 2 in Cardon and Bell 2001). Because of the low LD expected in natural populations and large breeding populations of trees, these associations will only be detected if the marker being tested is tightly linked to a QTL for the trait of interest. In other plants, LD ranges from about 200–1500 bp in maize (an outcrossing species) to 250 kb in *Arabidopsis* (a selfing species) (reviewed in Gaut and Long 2003). Furthermore, the extent of LD varies in different regions of the genome (reviewed in Tenaillon et al. 2001; Ardlie et al. 2002; Nordborg et al. 2002). Preliminary results from *Pinus taeda* suggest that LD is mostly 2500 bp or less (D.B. Neale and G.R. Brown, unpublished data). In *Pinus sylvestris*, no LD was observed among 12 single nucleotide polymorphisms (SNPs) within a 2045-bp fragment of the *pal1* gene (Dvornyk et al. 2002). Unlike pedigree analyses, association studies can be used to fine map and detect marker associations with QTL that are more likely to extend across families. Therefore, these markers might be useful for marker-assisted selection in typical breeding programs and for studying adaptation and genetic structure in natural populations.

Because only tightly linked markers will result in QTL–marker associations, genome-wide scans would require many markers to reliably detect most of the important QTL. Judson et al. (2002), for example, estimated that 180 000 – 600 000 carefully selected SNPs would be needed for a genome-wide scan in humans (i.e., human haplotype survey). If only gene-based functional regions are scanned, then 138 000 – 245 000 efficient SNPs are needed, the development of which would require genotyping of as many as several million SNPs.

In trees, particularly conifers, genome-wide scans seem unlikely; therefore, other approaches must be considered. One approach might involve scanning small portions of the genome, e.g., using markers in genomic regions known to contain important QTL. An alternative is to use candidate gene markers, rather than completely random markers or markers in randomly selected genes. Based on the assumption that these candidates represent the actual QTL of interest, this approach is not dependent on LD among loci. On the other hand, within-locus linkage equilibrium will complicate association studies in trees (i.e., requiring multiple SNPs per candidate), if LD is mostly less than 2500 bp (discussed above). For the rest of our discussion, we will assume that association studies in forest trees will be conducted using candidate gene markers and that the problem of imperfect linkage between marker and QTL can be ignored. To conduct association studies in forest trees, we will need to (*i*) identify candidate genes; (*ii*) find useful polymorphisms in candidate genes; (*iii*) develop study populations; (*iv*) phenotype and genotype individuals; and (*v*) verify associations using independent populations.

Identifying candidate genes

"Candidate gene" is used to denote a gene believed to have an important functional role in a trait based on indirect or circumstantial evidence. Because of our interest in understanding genetic variation in cold adaptation, we further define "candidate gene" as one that is plausibly responsible for phenotypic variation in natural or breeding populations. Promising candidates include genes with known functions, positions, or patterns of gene expression that are associated with the trait of interest.

Functional candidates can be identified via direct physio-

logical studies in trees or by DNA sequence homology to genes with known functions in other organisms. For example, pure physiological approaches and analyses of transgenic trees have directly implicated one or more of the phytochrome genes (*PHY*) in photoperiodism, bud set, and cold acclimation (Dinus 1968; Howe et al. 1996; Olsen et al. 1997*b*). *PHYB1* and *PHYB2* are particularly interesting, because genetic variation in (or near) these loci is readily detectable in black cottonwood, and comparable variation is not seen for *PHYA* (Howe et al. 1998). A great deal of effort is needed to obtain direct a priori physiological evidence. Although numerous candidate genes have been identified based on direct physiological studies (Howe et al. 1999; Rohde et al. 2000), these studies require much time and effort. Therefore, by using direct physiological approaches, we will only scratch the surface of the total number of genes (~30 000) that are expected to be found in forest trees.

Most candidates are likely to be identified via their homology to genes of known function in other organisms, such as *Arabidopsis*. We are using analyses of expressed sequence tags, for example, to identify candidate genes for cold hardiness in Douglas-fir. By comparing the DNA sequences of the Douglas-fir genes to gene sequences in data banks, we have already identified numerous candidates in our expressed sequence tag database (also discussed below). Because the complete genome sequence of black cottonwood (*Populus trichocarpa*) will soon be available (Wullschleger et al. 2002), we will have a substantial number of candidates from which to choose. These candidates might include cold hardiness genes such as the CBF/DREB1 family of transcriptional activators and the many genes that are induced during cold acclimation (Thomashow 1999, 2001), or genes involved in phytochrome signaling (Wang and Deng 2002), vernalization (Michaels and Amasino 2000), and phytohormone physiology (Xiong et al. 2002). One drawback of this approach is that most of the plants from which these genes have been isolated are annuals that could lack homologs for genes important for cold adaptation in woody perennials. This approach also presupposes that we can successfully infer a candidate's potential for explaining phenotypic variation in trees based on its DNA sequence and physiological function in other plants.

Positional candidate genes are those that map close to important QTL. Because of the large LD in typical QTL mapping experiments, however, the value of this information is limited, because these regions may contain hundreds or even thousands of genes. Nevertheless, position information is helpful for prioritizing genes that have already been deemed likely candidates based on other information. In poplar, for example, phytochrome genes (*PHYB1*, *PHYB2*) and abscisic acid insensitive genes (*ABI1B*, *ABI3*) are good candidates because they map near QTL for frost damage, bud set, or bud flush (Frewen et al. 2000; Chen et al. 2002). In Douglas-fir, we found candidate genes that mapped near cold hardiness QTL, including genes similar to those that encode a cold-inducible late-embryogenesis-abundant protein (LEA); prefoldin, a protein that regulates protein folding and protects proteins from freezing; ubiquitin, a protein that helps eliminate abnormal proteins following cold damage; a thaumatin-like protein precursor (antifreeze protein); a thiamin biosynthetic enzyme (TBE) that is responsive to water deficit; and LP3, a protein that is induced by water deficit in loblolly pine (D.B. Neale and K.V. Krutovskii, unpublished data). In each case, it would be logical to begin association studies with those genes that have been implicated as candidates by both sequence homology and map position.

Expression candidates are genes with patterns of gene expression that are associated with the trait of interest. This might include genes that are up- or down-regulated during cold acclimation and deacclimation, or in response to environmental signals such as short days or chilling treatments. DNA microarrays, which can be used to monitor the expression of thousands of genes at a time, are particularly powerful for screening large numbers of genes for the most promising candidates. In addition, gene expression analyses can be combined with other approaches to help reduce a large number of provisional candidate genes to a reasonable size. Microarray analyses were used to reduce the number of candidate genes in *Drosophila* from 548 (based on QTL localization and deletion mapping) to 34 genes that may influence ovariole number (Wayne and McIntyre 2002). Hybrid approaches, in which one searches for transcripts rather than SNPs that are associated with phenotypes, may also prove valuable. Oleksiak et al. (2002), for example, used a common-garden experiment to study gene expression differences within and among populations of minnows. They found significant differences in expression among individuals within populations (18% of genes), but only about 2% of the genes differed among populations. Although the population differences may result from random processes such as genetic drift, notable differences in gene expression were observed between the northern and southern populations. Therefore, the genes exhibiting these differences are good expression candidates for cold adaptation (Oleksiak et al. 2002). The drawbacks to this approach are that changes in gene expression may not be manifested at the mRNA level or may be difficult to detect if mRNA levels are low.

Finding useful polymorphisms in candidate genes

Once candidate genes are identified, the next step is to find variants that can be used as genetic markers. The current method of choice is to find SNPs, which are single base differences in DNA sequence. In loblolly pine, SNPs were found by amplifying and sequencing candidate gene fragments from the megagametophytes of 32 unrelated seed (D.B. Neale and G.R. Brown, unpublished data). The advantage of SNPs is that they are common; in loblolly pine, for example, one SNP was found every 91 bp in coding regions, and every 37 bp in noncoding regions (D.B. Neale and G.R. Brown, unpublished data). Alleles corresponding to multi-SNP haplotypes for candidate genes may be more useful in association studies than individual SNPs. Once such alleles can be distinguished, the next step is to determine their inheritance, map positions, and standard population genetic parameters (e.g., number of alleles, allele frequencies, percent heterozygosity), then test for LD with other loci. Candidate genes for cold adaptation have already been mapped in Douglas-fir, loblolly pine, and *Populus* (Frewen et al. 2000; D.B. Neale and G.R. Brown, unpublished data). Given that sufficient SNP variability can be found, a wide

range of candidate gene markers will soon be available for association studies.

Study populations

The choice of study population is critical for association studies. Associations should be studied in populations in which LD exists only between loci (or portions of loci) in close proximity to one another on the same chromosome (note, LD denotes the presence of correlated allele frequencies among two or more loci, regardless of whether the loci are physically linked or not; Falconer and MacKay 1996). Other causes of LD include the mixing of subpopulations with different allele frequencies, use of related individuals, genetic drift, and selection. Population admixture is unlikely to be a problem for most natural populations of forest trees, except in hybrid zones. Although related trees should not be used, we do not know what geographic distance should be maintained among trees chosen for association studies to ensure sampling from panmictic populations, while still avoiding closely related trees. The appropriate spatial scale may be population dependent, as genetic drift or mixed-mating can cause LD in small peripheral or disjunct populations (e.g., *Picea sitchensis*, W.J. Gapare and S.N. Aitken, unpublished data). In most situations (i.e., large, high-density, core populations), forest trees are highly outcrossed, gene flow via pollen can be extensive, and there seems to be little population differentiation for neutral genetic markers (Table 1). In contrast, selection is likely to cause among-population LD among loci that encode genes responsible for adaptation to complex environments. Douglas-fir trees that evolved near the Pacific Ocean, for example, are adapted to warmer temperatures and moister conditions than are trees from the more-inland Cascade Mountains, thus coastal and Cascade populations are likely to be in LD for loci conferring adaptation to these contrasting environments. If associations are found between cold hardiness phenotypes and allele frequencies for specific candidate genes in a pooled sample of individuals from these two areas, it will be difficult to judge whether these genes are responsible for enhanced cold hardiness, drought hardiness, variation in bud phenology, or some other trait that differs between these provenances. Patterns of adaptive genetic variation have been well studied by forest geneticists and are often used to delineate seed zone boundaries (reviewed in Morgenstern 1996). Therefore, it seems wise to use unrelated trees within seed zones (or some other population with sufficient genetic homogeneity) as the basic unit in any association study. This also has the potential advantage of integrating association studies with breeding programs.

Despite the advantages of focusing on within-seed-zone analyses, it would be desirable to sample multiple seed zones that span important adaptive clines for a number of reasons. First, false positive associations may be found within seed zones because of genetic drift or relatedness of trees (but see above). Second, alleles with large effects may be missed because they are rare in some seed zones, but common or even fixed in others. Third, associations that are repeatable across a wide range of environments are the most important to uncover. Finally, it would be valuable to know whether the same loci that vary within populations are also responsible for differentiation among populations, and ultimately, among species.

The power of association studies is influenced by both the size and composition of the population under study. For scans of individual candidate genes, populations of 500 or more unrelated individuals are probably needed to detect associations between SNPs and a quantitative trait (i.e., given that a quantitative trait nucleotide exists that explains 2.5%–10% of the phenotypic variance) (Long and Langley 1999). In contrast, empirical case-control studies of human diseases suggest that thousands of individuals might be needed to reliably detect associations (Cardon and Bell 2001). The simplest approach would be to genotype and phenotype a large number of unrelated individuals from one or more seed zones (described above). An alternative mapping strategy proposed by Wu et al. (2002) involves genotyping parents from a large randomly mating population and a sample of their open-pollinated progeny. This approach combines the advantages of pedigree linkage analyses and association studies to address the problem of not being able to distinguish strong association and loose linkage from weak association and tight linkage.

Natural populations would be best to use in association studies to avoid the influence of artificial selection on allele frequencies and LD. Nonetheless, it should also be possible to use genotypes from forest tree breeding programs; these breeding programs are young, and allele frequencies have changed little during the one or a few generations of selection that have occurred. One large advantage of using trees in breeding programs is that parents may have already been phenotyped in field or nursery progeny tests. In addition, the materials needed to conduct combined linkage analyses and association studies are probably available (Wu et al. 2002).

Phenotyping and genotyping

The ability to detect associations between phenotypes and candidate gene markers depends on the proportion of variation explained by a given locus and the precision of phenotypic measurement. Therefore, initial work should focus on high-heritability traits (such as bud phenology) and use common garden tests with large sample sizes that minimize environmental variation. Methods of phenotyping cold adaptation traits are well developed for forest trees. Useful test designs will include seedling nursery tests in which many important traits can be measured efficiently and with high heritabilities (e.g., O'Neill et al. 2001), tests in controlled environments (e.g., Howe et al. 2000), and long-term field tests that sample multiple sites and that can be used to examine both genotype × year and genotype × site interactions (Aitken and Adams 1996; Johnson 1997). Once found, SNPs can be used to genotype large numbers of individuals (e.g., parents or progeny from the genetic tests described above) using one of the many high-throughput approaches available (Nordström et al. 2000; Kwok 2001). An advantage of many conifer species is that the seed megagametophyte is haploid. Therefore, the genetic contributions from each parent (i.e., parental haplotypes) and the diploid genotype of the maternal parent can be easily inferred from a sample of seeds, even if the parent is no longer available.

Verification

Although associations may be found between candidate genes and phenotypes, these results are correlative. Other

methods will ultimately be required to establish cause and effect. The first step would be to verify the associations using other parents, populations, or species (Sewell and Neale 2000; Göring et al. 2001). Second, other correlative evidence can be mustered, including analyses of gene expression (Wayne and McIntyre 2002; Oleksiak et al. 2002) and testing for physical linkage (i.e., to remove other causes of LD as the source of associations) (Wu et al. 2002). Ultimately, however, genetic engineering approaches to alter the expression of introduced or endogenous alleles may be required. In poplar, genetic transformation is feasible and high-throughput approaches for analyzing hundreds of transgenes within a reasonable time appear realistic. In conifers, these studies are more difficult, because conifers are difficult to transform. Another option is to transform and study conifer genes in other species, but this may lead to ambiguous results.

Research needs and applications

Association studies may ultimately provide the tools for better understanding genecology, microevolution, and speciation, as well as for improving the efficiency of breeding programs. Nonetheless, this will only happen after we can reliably explain a reasonable proportion of the phenotypic variation among individuals and populations. Molecular approaches that cannot explain a substantial proportion of the phenotypic variance in cold adaptation (perhaps 20% or more) will probably have little impact on breeding programs, because classical approaches are likely to be more efficient. Because we expect that individual markers will only reliably explain a few percent of the phenotypic variation in natural and breeding populations, our long-term goal should be to develop robust multilocus approaches for explaining substantial proportions of genetic variation.

Prediction of individual phenotypes

Given the genetic complexity of cold adaptation, useful prediction of individual phenotypes will likely require the use of genotypic information at many loci, as well as information about the environments in which the genotypes are growing. A simple model that illustrates this concept follows:

$$[2] \qquad P_{ind} = f[\{G_1, G_2, \ldots, G_i\}\{E_1, E_2, \ldots, E_n\}]$$

where P_{ind} is the relative phenotypic performance of the individual having the multilocus diploid genotype $\{G_1, G_2, \ldots, G_i\}$ when tested in environments $\{E_1, E_2, \ldots, E_n\}$; G_i is the diploid genotype at the ith locus; and E_n is the environmental characteristics of the nth environment in which the genotype is growing. From a breeding perspective, one might be interested in predicting the average performance across all environments in which a genotype will be planted (e.g., $\{E_1, E_2, \ldots, E_n\}$ = single deployment zone). From a genecological perspective, one might be interested in predicting the performance of a genotype under specific environmental conditions, such as alternative photoperiodic, temperature, or moisture regimes (e.g., $\{E_1\}$, $\{E_2\}$, $\{E_n\}$ = separate environments).

Optimally, the function used to predict phenotypic performance will incorporate information on the average effects of alleles at each locus, allelic interactions (i.e., dominance or overdominance), and interlocus interactions (i.e., epistasis), as well as interactions between these genotypes and the environment (or environments) in which they are growing — a tall order! How does this long-term goal influence our assessment of future research needs? First, we should be prepared to screen many loci in our search for useful associations, pay careful attention to the many false positives that are likely to result from multiple testing, and develop rigorous tests for verification. Second, our association populations should have large numbers of individuals, probably in the thousands. Given the large number of main effects and interactions that will be needed to successfully predict phenotypes, over-parameterization of our prediction models will be a large problem if the number of trees sampled is too small. Finally, association populations must be planted across a wide range of environments to ensure that associations are either generalizable across environments or that the major genotype × environment interactions can be explained.

Prediction of population phenotypes

The goal of genecology is to understand the relationships between genotypes and the environments in which they grow. In many cases, we can reasonably predict the phenotypic performance of a population by knowing key characteristics about the environment in which it has evolved, with climatic information and surrogates for climatic information being the best measures of the environment (discussed above).

The prospect of finding useful associations between genetic markers and phenotypes suggests two possibilities for using marker information at the population level. First, can we predict the average performance of a population based on allele frequencies at multiple QTL? A simple model illustrating this concept follows:

$$[3] \qquad P_{pop} = f[\{F_{11}, F_{12}, \ldots, F_{ij}\}\{E_1, E_2, \ldots, E_n\}]$$

where P_{pop} is the relative phenotypic performance of the population having the multilocus allele frequencies $\{F_{11}, F_{12}, \ldots, F_{ij}\}$ tested in environments $\{E_1, E_2, \ldots, E_n\}$; F_{ij} is the frequency of the jth allele at the ith locus; and E_n represents the environmental characteristics of the nth environment in which the genotypes are growing. As discussed above, one might be interested in predicting the average performance of the population across a range of environments $\{E_1, E_2, \ldots, E_n\}$, or in a single, well-characterized environment $\{E_1\}$.

Second, can we relate the molecular genetic information about populations to the environments in which they have evolved and adapted? A simple model illustrating this concept follows:

$$[4] \qquad f[\{F_{11}, F_{12}, \ldots, F_{ij}\}\{E_1, E_2, \ldots, E_n\}] = f\{C_1, C_2, \ldots, C_n\}$$

where all terms are as described for eq. [3], except $\{C_1, C_2, \ldots, C_n\}$, which is the set of climatic or other environmental characteristics describing the environments in which the populations evolved.

Note that if we substitute P_{pop} for $f[\{F_{11}, F_{12}, \ldots, F_{ij}\}\{E_1, E_2, \ldots, E_n\}]$ in equation [4], we see that this approach is merely an extension of concepts commonly used to relate

multitrait population phenotypes to the characteristics of the environments in which they evolved. A common approach for doing this is to derive principle component scores for populations (i.e., using phenotypic traits measured in one or more test environments), then regress these on variables describing the environment in which the population evolved (e.g., Campbell 1986). Canonical correlation analysis has already been used to relate variation in multilocus allele frequencies to multiple characteristics of the environments in which the populations evolved (Westfall and Conkle 1992; Hamann et al. 1998). Westfall and Conkle (1992), for example, genotyped 158–516 trees at 10–32 allozyme loci, then used canonical correlation analysis to describe relationships between multilocus allozyme variation and geographic variables in four conifers from the Sierra Nevada Mountains of California.

Summary

Studies of cold adaptation in forest trees are proceeding to the molecular genetic level. Many of the key insights about cold adaptation at the quantitative and physiological genetic level have been amply demonstrated in numerous species, including both conifers and angiosperms. Traits responsible for cold adaptation have been identified, genetic and environmental correlations have been established, and geographic and environmental patterns of genetic variation have been described. What is missing is a detailed understanding of the genes that underlie these complex traits, how these genes vary among individuals, populations, and species, and how these genes contribute to fitness. Population genomics approaches, including association studies, should help increase our understanding of cold adaptation. This information, in turn, should help us better understand the evolution and ecology of forest trees, conserve the adaptive genetic variation needed for survival in current and future environments, and improve the efficiency of tree breeding programs.

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