True adaptation to a new environmental regime necessitates genetic change, or microevolution, to sustain the adaptation within a population. Apparent microevolution can sometimes be mimicked by phenotypic plasticity (e.g. Merilä et al. 2001), but plasticity does not represent a true evolutionary response to change. In order to determine if a population is truly adapting, the genetic component of phenotypic change must be untangled from the potential plastic response. The classical approach to calculating this genetic contribution comes from quantitative genetics, which are used to calculate the correlation between relatives and the response to selection using pure statistics or genetics based on allele frequencies (Gillespie 2004). More recently, molecular genetics have provided the tools to estimate the heritability of traits and thus the contribution of genetics to changes in phenotype. Both approaches depend on understanding the heritability of specific traits that are under selection, but the resolution of molecular genetics allows this analysis to be accomplished without the difficulty of conducting multi-generational studies. The following outlines the approaches employed in both quantitative and molecular genetics in discerning genetic contribution to adaptation and provides examples of these approaches in studies of natural populations.

*Quantitative Genetics*

Quantitative genetic approaches are used to determine the contribution of genetic heritability when there is a gradient of phenotypes in an environment. Phenotypic gradients could represent either a phenotypically plastic response to changes in the environment or true adaptation via inherited traits. The former acts within generations, whereas the latter acts between generations. Calculations of the heritability of specific traits provide the insight necessary to separate plasticity from microevolution. Heritability is the proportion of phenotypic variance due to additive effects and is defined by *h*2 = VA/ VP = VA /(VA + VE), where VA is the additive genetic variance, VP is the phenotypic variance, and VE is the environmental variance (Gillespie 2004). Most morphological and physiological traits are highly heritable with heritabilities greater than 10% and ranging to 60% in *Drosophila* (Roff & Mousseau 1987). Life history and behavioral traits, however, are more directly linked to fitness and tend to have heritabilities less than 10% (Roff & Mousseau 1987). Calculating heritability and genetic contribution to phenotype in this manner requires studies spanning multiple generations in a population so that the transmission of the phenotype can be followed from parent to offspring. Even after multiple generations of study, results can still be occluded by correlation of traits (Grant & Grant 1995) or incomplete understanding of how environmental variables affect phenotype.

The “animal model” has been used in a variety of studies of natural populations to provide clarity in assessing the plastic versus the microevolutionary response of a population. It is a mixed model that uses linear regression and explanatory terms of both fixed and random effects to estimate heritability (Kruuk 2004). The model is defined at the level of an individual animal and provides estimates of parameters from which heritability can be estimated using maximum likelihood (Kruuk 2004). The animal model necessitates an accurate pedigree with known parentage, which can be provided from observational or genetic data (Kruuk 2004).

Some of the more elegant applications of the animal model follow breeding pairs of animals over multiple generations to quantify plastic response to interannual climatic variation and over multiple generations to estimate heritability. Réale et al. (2003) observed female North American red squirrels (*Tamiasciurus hudsonicus*) from 1989 to 2001 and observed the females’ plastic responses of parturition date to annual spruce cone abundance (food resources) and their microevolutionary responses across generations through changes in estimated breeding values (the total additive effects of genes on a trait). The onset of spring has advanced in the squirrel’s range and the peak availabilities of spruce cones has followed suite, creating a selection pressure that could necessitate adaptation. Breeding day advanced 18 days over 10 years for the squirrels due in part to microevolution, which accounted for 0.8 days per generation, and plastic response to earlier abundances of cones (Réale et al. 2003). Partruition date in *T. hudsonicus* has significant genetic variation and is a heritable trait with *h*2 = 0.16 (Réale et al. 2003). The animal model was also used in a study of egg-laying date in great tits, *Parus major*, from 1973-2003 (Gienapp et al. 2006). The selection differential for predicted breeding values should be equal to the expected response to selection on phenotypes if environmental bias is excluded (Gienapp et al. 2006). Gienapp et al. (2006) found that there was strong selection at the phenotypic level for early breeding as measured by fitness, but there was no evidence that selection affected the evolution of the trait (i.e. there was no correlation between egg-laying date and fitness). The annual mean egg-laying date advanced 5.4 days over the study, but the evolutionary response was only 1.5 days over the 30 years, which was undetectable given the significant plastic response to the large environmental variation in breeding time (Gienapp et al. 2006).

Selection experiments in the lab are frequently employed in quantitative genetics to instigate the evolution of a trait and then measure its heritability in a common garden setting. These methods have been used in a study of the evolution of critical photoperiod in the pitcher plant mosquito, *Wyeomyia smithii*, and in the response of guppies, *Poecilia reticulata*, to relaxation of predation pressure. Mosquito larvae were collected throughout the species’ range from Florida to Ontario and bred to the F2 generation in the lab (Hard et al. 1993). The larvae were then separated into early and late diapausing groups by exposing them to increasing photoperiods. The heritability of critical photoperiod in the F3 generation was 0.3-0.7 (Hard et al. 1993). Critical photoperiod is the daylength at which the mosquito switches from active development to diapause. The genetic variance of the critical photoperiod trait is higher in the northern than in the southern populations, possibly due to more environmental variation in the north for the onset of favorable environmental conditions (Hard et al. 1993). The northern population also shows a more pronounced and faster microevolutionary shift in critical photoperiod over time, possibly due to stronger selection pressure and a greater capacity to evolve given the greater genetic variance (Bradshaw & Holzapfel 2001).

The guppy, *P. reticulata*, also showed differential microevolutionary response to selection pressure, but the difference was between sexes, not geographic locales. Guppies were introduced from high- to low-predation streams and after a number of years were reared in the lab to F2 and F3 generations. Age and size at maturity are highly heritable in *P. reticulata*, but more so in males than females, which allows the males to evolve more rapidly (Reznick et al. 1997). This could be because there are more biological restrictions on the development of female fecundity since in general it is more energetically costly to be female. Descendants of these transplanted fish matured at a later age and at a larger size than the source population and produced fewer, larger offspring per litter (Reznick et al. 1997). For both the guppies and the squirrels changes in natural selection patterns promoted microevolution and multi-generational studies allowed for the quantification of heritability.

*Molecular Genetics*

Molecular genetic techniques, including parentage analysis and genome-wide scans, can be used to detect signals of microevolution/adaptation without doing multi-generational studies. These approaches depend on specific patterns of inheritance of DNA markers that can be revealed with statistical techniques. Recently, mechanisms of adaptation have been discovered by pairing gene expression data (the functional phenotype) with DNA polymorphism data (microevolution). As technology evolves, it will become easier to discover the functional applications of changes in DNA sequence as microevolution occurs.

Parentage analysis, or pedigrees, is useful in estimating the heritability of a trait over one generation while accurately tracking true parent-offspring relationships. It is sometimes difficult to determine true pedigrees without molecular markers due to extra-pair matings. This type of analysis is similar to traditional quantitative genetic techniques in that multiple generations (in this case just parents and offspring) are needed and specific traits are measured in both generations. Darwin’s finches (*Geospiza* spp.) on the Galápagos islands, have been studied for many years and show significant heritability of morphological traits in response to environmental selection (Grant & Grant 1995; Grant & Grant 2006). Microevolution has been detected previously using quantitative genetics (Grant & Grant 2006) but the possibility of extra-pair matings made accurate estimates of heritability difficult. Keller et al. (2001) measured morphological traits and genotyped the parents and offspring of *Geospiza fortis* at microsatellite markers. They used regression analyses to estimate heritability and found that *h*2 of beak size and shape is 0.85 and 0.88, respectively. More than 85% of the phenotypic variation in these finches can be attributed to additive genetic variation (Keller et al. 2001). In this case, molecular genetics increased the accuracy of heritability estimates, reinforcing the important role of microevolution in the finches’ response to sudden environmental change.

Genome scans are a non-biased approach to discovering the genetic loci that are responsible for adaptation. Genome scans make use of multiple markers across a genome to discriminate outlier loci from normal genomic variation. The outlier loci show anomalous patterns of heritability or differentiation between populations that indicate the potential presence of selection pressures. A genome scan of the white spruce, *Picea glauca*, from six different ecological regions in Québec unveiled 49 single nucleotide polymorphisms (SNPs) that showed trends of local adaptation (Namroud et al. 2008). In this study, expressed sequence tag (EST) data were used to find the SNPs so that the markers would have a functional significance that would potentially underlie local adaptation. The candidate SNPs for adaptation were unevenly distributed across the populations and corresponded to genes that maintain development and growth in stressful environments, perhaps indicating that specific ecological regions promote local microevolution (Namroud et al. 2008). A genome scan of the common frog, *Rana temporaria*, across three altitudes yielded four loci that may be linked to adaptive differences (Bonin et al. 2006). The outlier loci were determined based on a confidence range from 20 different demographic scenarios, creating relatively conservative conditions for determining outlier status (Bonin et al. 2006). Altitude was responsible for 35.5% of the molecular variance in *R. temporaria* and the global FST over the altitudinal range was 0.11 (Bonin et al. 2006). Three of the candidate adaptive loci were monomorphic in at least one population, lending support to their importance in adaptation to regional climate regimes (Bonin et al. 2006).

Studies that combine physiological gene expression and DNA sequence data can elucidate the underlying mechanisms of adaptation to environmental change. The use of DNA sequence to understand microevolution is in actuality a secondary measure of the process of adaptation since the change has already been established. Gene expression and detailed analysis of function genes provide more of a real-time analysis of how adaptation to the environment is occurring and why fixed differences in the DNA sequence occur. Such analyses in Atlantic tomcod, *Micrgradus tomcod*, and the Glanville fritillary butterfly, *Melitaea cinxia*, uncover the mechanisms of species adaptation to novel local environments. Tomcod have been exposed to high levels of PCBs in the Hudson River since the late 1940s, creating a strong and localized selection pressure. This consistent selection has caused the Hudson River (HR) population to establish fixed differences in the sequence for an aryl hydrocarbon receptor (AHR), a receptor that is important in mediating toxicity of many environmental contaminants (Wirgin et al. 2011). The HR allele has one non-synonymous mutation, two synonymous, and on 6-base pair deletion compared to the allele found in neighboring uncontaminated populations (Wirgin et al. 2011). The 6-bp deletion seems to be the mutation that allowed for decreased effects of toxins on *M. tomcod* physiology since it decreases the binding affinity of AHR to PCBs (Wirgin et al. 2011). Neighboring populations still harbor this HR allele, but at low frequencies (Wirgin et al. 2011). Since there continues to be gene flow between the HR and proximal populations, the pollution tolerant allele was most likely at low frequencies in the population before the selection pressure of the pollution, which rapidly selected for the resistant allele.

The selection pressure in the Glanville fritillary butterfly population is colonization of novel environments within the *M. cinxia* metapopulation. Older populations and newly established populations were bred in the lab to F2 and then compared in a common garden experiment (Wheat et al. 2011). The new populations had higher expression of genes for reproduction (transfer of nutrients to eggs, egg provisioning protein, and oviposition) and protein turnover in muscle (proteasomes and chaperones; Wheat et al. 2011). These gene expression profiles could indicate a higher fitness of the newly established populations, which also have a higher peak metabolic rate during flight (Wheat et al. 2011). Specific alleles of *phosphoglucose isomerase* and *succinate dehydrogenase d*, which are implicated in flight metabolic rate, population age, and population growth rate, are associated with higher expression of the genes that are up-regulated in the new populations (Wheat et al. 2011).

Quantitative and molecular genetic approaches provide the tools necessary to understand the role of genetic change in response to environmental variability. Organisms across all taxa must constantly acclimatize or adapt as their ranges expand to new territory or as the environment changes within their established range. The interplay of microevolution and phenotypic plasticity has frequently compelled researchers to unravel their interaction. The recent accessibility of genome and trascriptome sequencing capabilities provide more resolution in understanding these dynamics. The greater clarity afforded by these new technologies will help lead to more in-depth discoveries of the processes of genome-environment interactions, including plasticity, microevolutionary adaptation, and speciation.

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