Question from Lorenz Hauser: *Genetic adaptations are difficult to separate from phenotypic flexibility, especially in wild populations.*

*a. Review approaches to verify that observed phenotypic changes have a genetic basis b. Discuss how genetic adaptation and phenotypic flexibility interact when populations are exposed to changing environmental conditions.*

In a population’s response to changing environmental conditions, changes in genotype (microevolution) and/or changes in the population’s mean phenotype (phenotypic plasticity) can contribute to adaptation. The distinction between the two mechanisms is not always discrete and they frequently interact during the process of adaptation. Phenotypic plasticity itself can be a heritable trait that is under selection, especially in variable environments or for phenological traits. Whenever evolutionary change in a population is investigated, contributions from variations in the reaction norm of the phenotype and from shifts in genotype frequencies should be considered. When the two interact, there need to be attempts to discern their relative contributions to evolutionary change and their potential for beneficial adaptation in a changing environment.

Shifts in the distribution of phenotypes during population adaptation can be caused by changes in genotype or phenotypic plasticity (Visser 2008). Plasticity is a highly beneficial trait in a variable environment where an organism’s ability to respond to seasonal and annual changes is linked to fitness. Flexibility in phenotype represents the scope of an organism’s or a population’s response to an environmental change. Since plasticity is a characteristic of a phenotype, it can also respond to selection pressures, but not in an evolutionarily significant way. Genetic adaptation is a more beneficial response evolutionarily when there is a directed environmental change. Phenotypic plasticity can be viewed in two ways: 1. As an ecologically important trait that can be selected for, or 2. As a developmental process that facilitates evolution (de Jong 2005). The former definition is the more functionally relevant and better supported by studies of natural systems. If phenotypic plasticity is a trait, then it can also be inherited and change in scope under different environmental pressures. The opposing mechanism to plasticity is canalization, or the buffering of a trait against genetic variation so that its important fitness contributions are not eroded (Wagner et al. 1997). Canalization can be disrupted under regimes of environmental change (Wagner et al. 1997) and so it most likely is a beneficial evolutionary trend in a relatively stable environment. Below I discuss how genetic adaptation, or microevolution, can be separated from phenotypic plasticity in studies of wild populations under changing environmental conditions through a review of the current literature. In different cases, genetic and plastic changes may interact, plasticity may have no role at all, or plasticity may be the genetic trait under selection. The interaction between genetic adaptation and flexibility in phenotype will probably be the most important interaction for a number of natural populations in a changing environment.

It is difficult to provide concrete evidence of a genetic basis to phenotypic change, but a variety of recent studies across a broad range of organisms solidly support or refute the role of genetic change in phenotypic shifts. When a genetic basis to change is lacking, support for the role of phenotypic flexibility is verified. For genetic change to be responsible for phenotypic change, the selected trait must be heritable. Also, the selection pressure must be strong and directed enough to select for one genotype/phenotype over another. In the classic example of Darwin’s finches in the Galápagos, environmental change and interspecific competition both contribute to change in beak size in *Geospiza fortis* (Grant & Grant 2006). After a drought on Daphne Major in 1977, *G. fortis*’ preferred small seeds were less available, so larger individuals had greater fitness and the mean beak size of the population increased. Another, larger finch, *G. magnirostris*, arrived on the island in 1982 and by 2003, its population numbers had grown to the point where it was able to outcompete *G. fortis* for large seeds (Grant & Grant 2006). This created an opposite selection pressure on *G. fortis* in 2004 and subsequent offspring had much smaller beaks, a highly heritable phenotype. In both 1977 and 2004, the respective selection pressures caused major declines in *G. fortis* populations before population mean phenotype changed and allowed a rebound (Grant & Grant 2006).

Phenotypic change similar to *G. fortis’* in response to environmental change is evidenced in the pitcher plant mosquito, *Wyeomiy smithii*, in North America. Larval diapause is determined by photoperiod in *W. smithii* and critical photoperiod, a heritable trait, signals the onset and maintenance of diapause (Bradshaw & Holzapfel 2001). This species experiences a differential shortening of critical photoperiod and has different levels of genetic diversity throughout its range. There was a greater shift towards shorter photoperiods in the north than the south from 1972 to 1996, which is also the part of the species’ range with the greatest genetic diversity for the photoperiodic response (Bradshaw & Holzapfel 2001). In *W. smithii*, populations in the northern part of the range exhibited a greater evolutionary response (manifested by change in critical photoperiod) to environmental change from stronger directional selection and a greater capacity to evolve (Bradshaw & Holzapfel 2001).

The examples of Darwin’s finches and the pitcher plant mosquito demonstrate potential for rapid response of microevolution in natural populations, a trend also seen in the guppy, *Poecilia reticulata*. Like *W. smithii*, *P. reticulata*, populations evolve at different rates under selection, but the rates are different between sexes within the same population (Reznick et al. 1997). Age and size at maturity as well as parental investment in offspring are heritable traits in guppies and all of these traits shifted when *P. reticulata* were transferred to habitats with decreased predation pressure (Reznick et al. 1997). After 11 years, maturation occurred at greater age and size for adult guppies and they produced fewer, larger offspring. Males evolved these traits faster than females due to differences in heritability between sexes since females had lower genetic variability for age and size at maturity than males (Reznick et al. 1997). The established heritability of these traits indicates that genetic change did occur with the relaxation of predation pressure, however there is also the possibility that males are more plastic for these traits than females and that female fitness depends more on being a certain size (i.e. females may exhibit genetic canalization).

It may frequently be the case in nature that phenotypic plasticity and genetic adaptation co-occur and can be easily confused with each other. The examples of northern cod and red squirrels address the problem of confounding changes in a population’s reaction norm in comparison to true evolutionary change. Northern cod, *Gadus morhua*, were the subject of a long-term directed fishery in the North Atlantic until the fishery crashed in the late 1980s. Olsen et al. (2004) discovered that the extensive fishing pressure selected for an evolutionary change in body size in cod. Fishing selected for early- and small-maturing genotypes, but the moratorium on fishing seemed to cause a relaxation in the selection pressure in 1993 (Olsen et al. 2004). The analysis leading to this conclusion took into account the reaction norm of the cod, the actual finding being that the reaction norm itself had decreased in the 1980s until 1993 (Olsen et al. 2004). In the case of cod, the entire distribution of phenotypes shifted over the years as consistent selection pressure favored females that reproduced before they were fished. Réale et al. (2003) also took into account both genetic change and phenotypic plasticity in their analysis of northern red squirrels, *Tamiasciurus hudsonicus* and their response to increased spring temperatures. Female squirrel reproductive behavior was monitored across breeding seasons and between generations of known pedigrees along with food availability (Réale et al. 2003). There was a strong effect of increased food availability on the plasticity of breeding date, but there was also strong selection for early breeders. Not only did females breed earlier in accordance with food availability across seasons (plasticity), subsequent generations of squirrels became earlier breeders via microevolution (Réale et al. 2003). Both flexibility of phenotype and genetic change resulted in adaptation to environmental change in keeping synchrony with a food source.

The heritability of a trait and its evolutionary change can sometimes be masked by greater pressures from the environment. A long-term study of great tits, *Parus major*, assessed the potential interaction of changes in phenotype and genotype in response to environmental change. Since 1973, the mean daily spring temperature has increased in the UK, along with the advancement of peak caterpillar biomass, which is the main food source for fledglings. *P. major*, however, did not change its laying date in kind, increasing the asynchrony between food availability and fledglings (Visser et al. 1998). This lack of response could be because the environment did not change uniformly and was not warmer when *P. major* decided to lay eggs (Visser et al. 1998). In a study of the same population, following females that lay in more than one year, Nussey et al. (2005) found that there is significant plasticity in laying response and females will lay earlier after a warm spring. There is significant inter-female difference in laying date and in magnitude of response to spring temperature, supporting that there is genetic variation and heritability for laying date plasticity (Nussey et al. 2005). Females with a more plastic response bred in closer synchrony with caterpillar peak biomass and had greater fitness as measured by offspring reproductive success (Nussey et al. 2005). Over 31 years of studying the *P. major* population, egg laying day advanced only 1.5 days (Gienapp et al. 2006). This microevolution was hard to detect because of the highly flexibly response of breeding to environmental variation (Gienapp et al. 2006). The strength of selection was also reduced by high adult survival (i.e. no differential mortality); sex limitation in only being able to measure the trait in females and unknown paternity; and low heritability of the egg-laying phenotype (Gienapp et al. 2006). In great tits, phenotypic plasticity plays a more significant role in successful reproduction and fitness, but the heritability of that plasticity is important for its maintenance in the population. Over time, even the plastic response was not enough to maintain synchrony with the environment, and microevolution has occurred, but its effect is so small relative to the phenotypic environmental response that it is almost undetectable.

Phenotypic plasticity and genetic change do not always co-occur during environmental change and when they do, plasticity can either help or inhibit microevolution, depending on a variety of factors. The interaction of phenotypic plasticity and genetic adaptation depend on the degree and consistency of environmental change and the phenotypic and genetic variation of the trait(s) under selection. In natural populations, the interaction can also be influenced by range shifts or immigration. Darwin’s finches, *Geospiza* spp., and the collared flycatcher, *Ficedula albicollis*, provide examples of potential for interaction between phenotypic flexibility and genetic change. Two environmental selection events, droughts in 1976-1977 and 1984-1986, inflicted opposing selection pressures on *Geospiza* spp. on Daphne Major (Grant & Grant 1995). Both environmental events created selection pressures on six highly correlated traits relating to body and beak size (Grant & Grant 1995). For the 1976 drought, all six measurements increased in the population, however for the 1984 drought there was not the same cooperative shift of traits due to shifts in available food (Grant & Grant 1995). Major environmental fluctuations caused microevolution in both cases, but a secondary shift in nutritional regime for the 1984 event resulted in an additional plastic response and deviation from the expected phenotype.

Two examples from the collared flycatcher underline the importance of phenotypic plasticity in environmental response and its ability to mask microevolution. *F. albicollis* fledgling relative body mass, a heritable trait, is an indication of survival and is under significant positive directional selection and weaker, less consistent stabilizing selection (Merilä et al. 2001). When both of these types of selection influence a heritable trait, they typically lead to genetic adaptation and microevolution (Visser 2008). In the case of flycatcher fledglings, however, the mean value for this trait has decreased despite selection pressures from 1981-1999 (Merilä et al. 2001). The cause of this counterintuitive trend is most likely countergradient variation: a negative covariance between environmental and genetic influences masking genotypic change (Conover & Schultz 1995; Merilä et al. 2001). The microevolutionary response to selection is hidden by the phenotypic response to environmental effects, which are most likely due to asynchrony with food sources (Merilä et al. 2001). Also in *F. albicollis*, significant changes in laying date in accordance with the North Atlantic Oscillation index is a purely plastic response (Przybylo et al. 2000). *F. albicollis* has probably evolved to compensate for the NAO changes by maintaining a large amount of plasticity in its choice of annual laying date. Future work may demonstrate that this plasticity is heritable and may have the potential to shift as it has in great tits (Gienapp et al. 2006).

The importance of phenotypic plasticity in evolution depends on the trait and the population’s historic interactions with its environment. Without plasticity, populations suffer large crashes during rapid evolution, as seen in Darwin’s finches (Grant & Grant 2006). The finches live in a relatively closed environment with small census population sizes and so are more able to recover from such crashes than some other species may be. In general, traits that depend on environmental variation for their timing, e.g. phenological traits involved in reproduction, will most likely have a greater degree of phenotypic flexibility and this flexibility may itself be under selection. In cases of rapid environmental change, genetic adaptation can sometimes be too slow and two instances of phenotypic plasticity would allow a population to adapt quickly: learning and maternal effects (Visser 2008). However, these reactions will no longer be adaptive if there is an uncoupling of environmental variables with the selected traits, which may very well be the case in the face of unanticipated climate change (Visser 2008). For management and conservation purposes, it will become more pressing to understand the adaptive potential of natural populations, a characteristic that includes plasticity and genetic variation. As demonstrated by a number of the studies described above, the interplay of plasticity and microevolution can only be understood with long-term and pedigree-based data sets. Since global climate change has already begun, it is important to identify representative at-risk species and begin these long-term studies now before the limits of their adaptive responses are exceeded.

Emma, same as the other question, it is difficult to follow your train of thought without any guiding subheadings. You review the literature, but you concentrate on the results of case studies and do not really comment on approaches verifying the genetic basis of phenotypic change. In all your examples, you don’t say how the authors verified that the change had a genetic basis or not, and that was really the main point of the first question.

The second part of the question is better, as you do discuss the interaction between phenotypic flexibility and genetic adaptation. I would have expected a more physiological point of view from somebody in your field, but as I was not specifically asking for that, it’s fine.

**References**

Bradshaw, W.E., and C.M. Holzapfel. 2001. Genetic shift in photoperiodic response correlated with global warming. *PNAS*. 98(25):14509-14511.

Conover, D.O. and E.T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. *TREE*. 10(6): 248-252.

Gi Gienapp, P., E. Postma, and M.E. Visser. 2006. Why breeding time has not responded to selection for earlier breeding in a songbird population. *Evolution*. 60: 2381-2388.

Grant, P.R. B.R. Grant. 1995. Predicting microevolutionary responses to directional selection on heritable variation. *Evolution*. 49(2): 241-251.

Grant, W.S. and B.W. Bowen 2006. Living in a tilted world: climate change and geography limit speciation in Old World anchovies (*Engraulis*; Engraulidae). *Biological Journal of the Linnean Society*. 88: 673-689.

de Jong, G. 2005. Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytologist*. 166(1): 101-118.

M Merilä, J., L.E.B. Kruuk, and B.C. Sheldon. 2001. Cryptic evolution in a wild bird population. *Letters to Nature*. 412: 76-79.

Nussey, D.H., E. Postma, P. Gienapp, and M. E. Visser. 2005. Selection on heritable phenotypic plasticity in a wild bird population. *Science*. 310(5746): 304-306.

Olsen, E.M., M. Heino, G.R. Lilly, M.J. Morgan, J. Brattey, B. Ernande, and U. Dieckmann. 2004. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Letters to Nature*. 428: 932-935.

Réale, D., A.G. McADam, S. Boutin, and D. Berteaux. 2003. Genetic and plastic responses of a northern mammal to climate change. *Proc.R.Soc.Lond.B.* 270(1515): 591-596.

Reznick, D.N., F.H. Shaw, H. Rodd, and R.G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulala*). *Science*. 275(5308): 1934-1937.

Visser, M.E., A.J. van Noordwijk, J.M. Tinbergen, and C.M. Lessells. 1998. Warmer springs led to mistimed reproduction in great tits (*Parus major*). *Proc Biol. Sci.* 265(1408): 1867-1870.

Visser, M.E. 2008. Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proc. R. Soc. B.* 275(1635): 649-659.

Wagner, G.P., G. Booth, and H. Bagheri-Chaichian. 1997. A population genetic theory of canalization. *Evolution*. 51(2): 329-347.