MINI REVIEW

Adaptation to environmental stress: a rare or frequent driver of speciation?

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Abstract

Recent results of evolutionary genomics and other research programmes indicate an important role for environment-dependent selection in speciation, but the conceptual frameworks of speciation genetics and environmental stress physiology have not been fully integrated. Only a small number of model systems have been established for cross-disciplinary studies of this type in animals and plants. In these taxa (e.g. *Drosophila* and *Arabidopsis/Arabis*), studies of the mechanistic basis of various stress responses are increasingly combined with attempts to understand their evolutionary consequences. Our understanding of the role of environmental stress in speciation would benefit from studies of a larger variety of taxa. We pinpoint areas for future study and predict that in many taxa 'broad' hybrid zones maintained by ecological selection will be valuable venues for addressing the link between environmental stress, adaptation, and speciation.

Introduction

Since environmental stress is notoriously associated with population decline and extinction, its potentially positive roles in evolution are often overlooked. Students of speciation often tend to ignore the concept of 'stress' or physiological response to it (maintenance of homeostasis through change; Romero, 2004). This is unfortunate since stressful environments have long been known to be associated with bouts of directional selection (Hoffmann & Parsons, 1997), and an increasing number of authors view selection as the primary cause for the origin of phenotypic novelty (e.g. reviews by Hoekstra *et al.*, 2001; Merilä & Crnokrak, 2001; Rieseberg *et al.*, 2002). One might even argue that other types of stress (other than exogenous environmental stress) have been more strongly if not sometimes over-emphasized in the speciation literature, e.g. intrinsic 'genetic stress' caused by hybridization, often thought to lead to the reinforcement of reproductive barriers (Dobzhansky, 1937; Butlin, 1989; Servedio & Noor, 2003), or 'genomic stress'

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caused by chromosomal change or polyploidization (McClintock, 1984; Wendel, 2000; Baumel *et al.*, 2002; Soltis *et al.*, 2004). This may be the case because, for most study organisms, intrinsic genetic or chromosomal factors have been more amenable to 'reductionist' laboratory studies than exogenous environmental stresses. Also, it is easier to include intrinsic factors in predictive models of speciation (Dobzhansky, 1937; Noor *et al.*, 2001; Navarro & Barton, 2003). Indeed, among the three potentially 'positive' contributions of stress to speciation outlined in Table 1, the role of exogenous environmental stress in divergence has perhaps been considered the least.

Here, we focus on the potentially positive roles of exogenous environmental stress in evolution, considering mainly abiotic and some of the better studied biotic environmental stresses (e.g. herbivory). We are aware that extinction by itself may in theory represent a positive outcome of environmental stress by creating open niches, i.e. novel opportunities. However, in the light of the present extinction wave (Wilson, 1992), we choose not to include extinction in our definition of 'positive'. Rather, we focus on the origin of phenotypic novelty through speciation or adaptive divergence at the *within*-species level. The latter scenario differs from the former in that it does not necessarily imply that populations are reproductively isolated, e.g. divergent natural

Table 1 Potential 'positive' roles of three types of stress in speciation.

Type of stress	Potential role in speciation	Further reading in the speciation/ organismal literature
Exogenous environmental stress	May lead to ecological divergence via environment-dependent selection associated with divergent habitats or niches	Templeton (1981), Schluter (2000) and Levin (2000)
Endogenous genetic stress/ outbreeding depression	May contribute to the evolution of reproductive barriers following hybridization between divergent populations	Dobzhansky (1937), Butlin (1989) and Servedio & Noor (2003)
Genomic stress caused by chromosomal change or polyploidization	May lead to the origin of phenotypic variation through genomic re-organization, gene silencing, and/or the release of transposable elements	McClintock (1984), Wendel (2000) and Soltis et al. (2004)

selection may still be in the process of driving the accumulation of gene flow barriers between subspecies or ecotypes at the time of study. However, the inclusion of speciation *and* adaptive population divergence allows us to draw from an enlarged pool of relevant studies.

We start our review by outlining some recent findings on the role of divergent selection and ecological divergence in speciation. This is relevant to the over-all topic, since accumulating evidence for a general role of natural selection in speciation motivates us to explore the role of environmental stress in speciation. We proceed by reviewing selected key studies on the role of environmental stress in adaptive divergence or speciation in both animals and plants, including recent methodological advances. Finally, we highlight how studies of speciation genetics and environmental stress response could potentially be combined. Our examples from plants may be more detailed than those from animals, since both of us are botanists, which may complement other contributions to this special issue.

The role of divergent selection in speciation – methodological aspects and recent experimental evidence

Whether or not natural selection plays a vital role in creating/maintaining species differences (Darwin, 1859; Grant, 1966; Templeton, 1981) has recently been addressed using a range of different approaches, including phenotypic selection experiments at the *within-* and *between*-population level (reviews by Hoekstra *et al.*, 2001; Lexer *et al.*, 2003a), quantitative trait locus (QTL) studies (Rieseberg *et al.*, 2002), comparisons of genetic divergence at neutral marker loci and quantitative traits (Merilä & Crnokrak, 2001; McKay & Latta, 2002), population genomic studies searching for the locus-specific footprint of selection (Wilding *et al.*, 2001; Campbell & Bernatchez, 2004) or combinations of this approach with QTL mapping (Rogers & Bernatchez, 2004), and tests for differences between synonymous

vs. nonsynonymous substitutions at protein-coding sequences (Nielsen, 2001). Below, we briefly introduce the concepts of these different approaches.

The phenotypic selection experiments reviewed by Hoekstra *et al.* (2001) and Lexer *et al.* (2003a) generally employed a multivariate approach that allows associations between individual phenotypic traits and fitness to be detected (Lande & Arnold, 1983; Phillips & Arnold, 1989), facilitating studies of directional selection (shifting the means of phenotypic characters), stabilizing/disruptive selection (affecting trait variances), or correlational selection (affecting trait correlations). The main difference between *intra*-population studies (Hoekstra *et al.*, 2001) and *inter*-specific selection experiments (Lexer *et al.*, 2003a) is that the latter permit studies of characters that may be invariant (fixed) *within* populations or species, but that may nevertheless be vital to understanding the factors that maintain species differences, or the forces that created these differences in the first place. The re-analysis of QTL data across kingdoms conducted by Rieseberg *et al.* (2002) followed a different approach. This method uses the directions (signs) of QTLs to make inferences about the evolutionary forces that created the phenotypic differences between two lines or populations. The underlying rationale is that traits with a history of directional selection will have QTL effects mostly in the same direction, whereas QTLs with effects in opposite directions should be common for traits diverging under neutrality (Orr, 1998).

Comparisons of genetic divergence at neutral markers and quantitative traits (Merilä & Crnokrak, 2001; McKay & Latta, 2002), on the other hand, are based on the prediction that traits experiencing strong selection in local environments will be more divergent than neutral markers. Similarly, population genomic studies on the role of selection in speciation (Wilding *et al.*, 2001; review by Luikart *et al.*, 2003; Campbell & Bernatchez, 2004) follow the premise that marker loci linked to genes under selection will display greater levels of genetic divergence (e.g. F_{ST}) than the remainder of the genome.

Finally, tests for differences between synonymous vs. nonsynonymous substitutions are a reliable method for studying positive selection at the sequence level (Nielsen, 2001), but adaptive changes in regulatory regions or noncoding RNAs will not be detected by this method (Schlötterer, 2003).

The overarching signal from the above studies/ approaches is that environment-dependent selection does play a crucial role in population divergence and speciation, although the strength of selection appears to differ between types of characters (e.g. stronger selection on life history traits, weaker selection on morphological traits; Rieseberg *et al.*, 2002; Lexer *et al.*, 2003a). Species differences are more likely to have resulted from selection than differences between conspecific populations (Rieseberg *et al.*, 2002), and the selection pressures that drive adaptive radiation and/or ecotypic differentiation appear to cause divergence at only few genetic loci while most of the genome is homogenized by gene flow (Wilding *et al.*, 2001; Campbell & Bernatchez, 2004; Rogers & Bernatchez, 2004), as predicted under the 'genic' view of speciation (Wu, 2001).

Stress, adaptation, and speciation in *Drosophila* and other animal 'model' taxa

Considering the important role of divergent selection in speciation, it is surprising that the concept of 'stress', or of physiological response to it as defined in the first paragraph of this paper, has only rarely been integrated into research on speciation/species barriers. Admittedly (from the perspective of the plant scientist), this appears to have happened more thoroughly in animal than plant models. For instance, responses to stress regimes imposed by aridity and solar radiation have been implicated in adaptation-driven incipient speciation in *Drosophila melanogaster* in 'Evolution Canyon', Israel (Michalak *et al.*, 2001). In this particular case, adaptation to contrasting microclimates appears to be associated with genetic divergence in the regulatory region of *hsp70Ba*, the gene encoding for the major inducible heat shock protein of *Drosophila* (see Hoffmann *et al.*, 2003, for a review of temperature stress response in *Drosophila*, and Sorensen *et al.*, 2003, for the evolutionary role of heat shock proteins). In another recent example, precise gene replacement was used to demonstrate the role of genetic variability at the *desaturase 2* gene locus of *D. melanogaster* in cold adaptation during incipient speciation in the fly (Greenberg *et al.*, 2003).

An interesting approach has recently been proposed for studying the molecular basis of adaptation in wild populations (Schlötterer, 2002a,2003), and a thorough evaluation in *Drosophila* and other animal taxa indicates that this method has a high potential in many species with similar population structures. In this approach, many molecular markers (such as microsatellites) with known genomic locations are assayed in many widely dispersed populations of a species, and strongly reduced genetic variability for specific markers/populations may be used as evidence for positive selection associated with local adaptation ('selective sweeps'; Schlötterer, 2002a,2003). Suitable genetic diversity statistics for this purpose, taking variation in mutation rates and demographic history into account, have been developed (Schlötterer, 2002b), and it has recently been shown that this 'hitchhiking mapping' approach was capable of detecting a beneficial mutation in the *Cyp6g1* gene of *D. melanogaster* conferring resistance to DDT (Catania *et al.*, 2004). An important aspect is that this methodology may also be applicable to *inter*-specific adaptive differences relevant to speciation if divergence took place relatively recently, provided that confounding factors are taken into account, e.g. periods of reduced effective population size (bottlenecks) that would reduce levels of allelic diversity in the neospecies. However, to our knowledge the true potential of the 'hitchhiking mapping' approach for studying selection pressures during speciation has not yet been evaluated, despite the fact that selective sweeps have long been thought to contribute to divergence during speciation (Hilton *et al.*, 1994).

Despite the predominant use of *Drosophila* in stress research, a role of environmental stress as an evolutionary force also becomes apparent in numerous other taxa beyond traditional model systems, e.g. mammals (Nevo *et al.*, 2000), amphibians (Rasanen *et al.*, 2003), or molluscs (Johannesson, 2003). The last example, focusing on zones of overlap between divergent *Littorina saxatilis* morphs, proved to be particularly fruitful. Populations of these snails occur on marine rocky shores strongly exposed to abiotic stresses such as shifts in temperature, salinity, and wave action (Johannesson, 2003). Reciprocal capture-recapture experiments indicated an important role for spatially varying selection in different locations where the two morphs of these snails co-occur (Janson, 1983; Johannesson *et al.*, 1997). Recent population genomic studies suggest the probable genetic basis of adaptation to these stressful and divergent habitats – only few genetic loci seem to be required (Wilding *et al.*, 2001). Clearly, students of animal systems are increasingly becoming aware that stress can sometimes be a positive factor in evolution, as also indicated by several other articles in this special issue.

Stress response in plants – always two ways to look at it?

In plants, increasingly severe biotic and abiotic stress regimes in agricultural habitats (e.g. salinity, drought) have led to considerable efforts to unravel the cascade of molecular events involved in stress response (e.g. reviews by Hasegawa *et al.*, 2000a,b; Knight, 2000). However, in-depth studies of the mechanistic basis *and* evolutionary significance of environmental stress response in plants are rare, despite the early observation that heavy metal

stress in plants may trigger adaptive changes leading to speciation (Macnair, 1983). It appears that research on the role of stress in plants often goes down only *one* of two possible roads (one leading to the unravelling of the molecular mechanisms involved, the other one to understanding its evolutionary consequences). This was often the case in the past, most likely because a joint analysis of the molecular pathways leading to stress response *and* assessing their capacity for (or constraints to) evolutionary change seemed difficult (White, 2001). However, new research concepts involving evolutionary and ecological functional genomics (Feder & Mitchell-Olds, 2003) within the framework of comparative biology increasingly allow these two different trajectories to be connected. Plant genera in which this is already yielding first results include *Arabidopsis/Arabis* and *Helianthus*.

Arabidopsis/Arabis – a field botanist's most underrated herbs

Arabidopsis thaliana and its wild relatives, e.g. *A. lyrata*, *A. halleri* and *Arabis* spp., have evolved a broad range of life-history, developmental and physiological adaptations to diverse and stressful habitats such as sand dunes (*A. lyrata*), heavy-metal contaminated sites (*A. halleri*), deserts/xeric grasslands (North American *Arabis* spp.), or high-elevation sites (other *Arabis* species; Mitchell-Olds, 2001). Also, Arabidopsis and *Arabis* spp. lend themselves to evolutionary studies: the predominantly selfing breeding system of *A. thaliana* and *A. drummondii* facilitates QTL analyses in advanced generation crosses, whereas the outcrossing *A. lyrata* and *A. halleri* lend themselves to population genetic analyses that rely on the assumption of random mating (Mitchell-Olds, 2001). Studies of environmental stress, adaptation, and evolution in this group have employed diverse experimental approaches such as common garden studies (McKay *et al.*, 2001), QTL mapping (Weinig *et al.*, 2003), expression profiling (Seki *et al.*, 2001), linkage disequilibrium (LD) mapping (Olsen *et al.*, 2004) and population genetic analyses of candidate genes (Clauss & Mitchell-Olds, 2003; Bishop *et al.*, 2000). Perhaps most remarkably, studies on *Arabidopsis/Arabis* have demonstrated that chitinase genes (coding for plant defence proteins that attack herbivores) underwent rapid adaptive sequence evolution as indicated by rates of nonsynonymous vs. synonymous base substitutions (Bishop *et al.*, 2000), that pleiotropy between a dehydration avoidance trait (carbon isotope discrimination) and a drought escape character (flowering time) facilitates drought tolerance in *A. thaliana* (McKay *et al.*, 2003), and that the genetic basis of ecologically relevant variation (e.g. flowering time) in this species may be elucidated by LD mapping (Olsen *et al.*, 2004).

The study of Olsen *et al.* deserves particular attention, since QTL and genetic association studies in natural populations have been suggested as a potential means of identifying genetic factors of evolutionary interest *and* assessing their fitness effects in the same experiment (Rieseberg & Buerkle, 2002; Slate, 2004). Also, the potential of LD mapping in organisms with different breeding systems and population structures has been hotly debated (Long *et al.*, 1998; Thornsberry *et al.*, 2001; Nordborg *et al.*, 2002), a particular concern in *A. thaliana* being that, due to its inbreeding mating system, the physical length of haplotype blocks in the genome could be a barrier to localizing causal polymorphisms (Nordborg *et al.*, 2002; Olsen *et al.*, 2004). Indeed, levels of LD in the genomic region of the photoperiod receptor *CRY2* in *A. thaliana* (Olsen *et al.*, 2004) indicated that the individual marker analyses employed in outbreeding species such as *Drosophila* (Long *et al.*, 1998) or maize (Thornsberry *et al.*, 2001) are less appropriate in *A. thaliana*, and that a haplotype-based approach, similar to LD mapping techniques used in human genetics, may be more successful in this species. With respect to evolutionary implications, the peculiar haplotype structure observed in the *A. thaliana* mapping population (two distinct haplogroups suggesting independent evolutionary origins), and the characteristic geographic distribution of haplotypes (correlations between haplotypes and mean January temperatures), are suggestive of adaptive evolution at these flowering time loci (Olsen *et al.*, 2004). This example was chosen because it illustrates the potential of LD mapping for genetic analysis of traits potentially involved in stress response (drought escape in the case of flowering time in *Arabidopsis thaliana*; McKay *et al.*, 2003; Olsen *et al.*, 2004).

In summary, *Arabidopsis* and *Arabis* species represent exciting model systems for studying many aspects of the role of stress tolerance in adaptive evolution. However, studies of a larger variety of taxa may benefit our understanding of the role of stress in speciation. Studies of taxa with mixed or outbreeding mating systems would be particularly desirable, especially in cases in which neospecies underwent truly remarkable ecological transitions involving new and stressful environments. Below, we review one selected example. Our choice was biased by the fact that one of us had the opportunity to contribute to the current state of knowledge in this genus.

Hybrid speciation and ecological transitions in wild sunflowers (*Helianthus*)

In *Helianthus* (sunflowers), adaptation to novel and extreme environments is closely associated with the origin of three diploid hybrid species, *H. anomalus* (sand dunes), *H. deserticola* (desert floors), and *H. paradoxus* (salt marshes; Rosenthal *et al.*, 2002), and it has recently been shown that the ecological transitions required for entry into these entirely new and stressful niches have been facilitated by hybridization (Rieseberg *et al.*, 2003). So

far, the best studied of these three cases is the salt-tolerant *H. paradoxus*.

Early growth-chamber studies of ecological divergence in this species revealed that *H. paradoxus* was more than five times as fit as either of its parental species under sodium chloride stress, and that a manageable number of heritable candidate traits was likely to be involved in this stress response (Welch & Rieseberg, 2002). In the next step, early generation backcross (BC$_2$) hybrids between the two parental species were transplanted into the salt marsh habitat of the natural hybrid species, *H. paradoxus*, in an attempt to 'replicate' the earliest steps of the hybrid speciation process (Lexer *et al.*, 2003b). This revealed strong directional selection on some of the candidate traits, including several mineral ion uptake characters that were expected to contribute to salt adaptation based on the salt stress literature (e.g. Flowers *et al.*, 1986; Cheeseman, 1988). Phenotypic variances for many candidate adaptive traits in the hybrid population were larger than in samples of the two parental species grown in the salt marsh, which may provide the raw material upon which ecological selection can act. Also, for most traits several hybrid individuals had extreme (transgressive) trait values compared to the two parental species (Lexer *et al.*, 2003b).

A QTL mapping study on the same field-grown backcross hybrids (Lexer *et al.*, 2003c) revealed the likely genetic basis for these phenotypic patterns: for each trait, QTLs with opposing effects were detected in the two parental species, which is exactly the genetic architecture required for 'transgressive segregation', i.e. the release of 'cryptic' variation through complementary gene action (deVincente & Tanksley, 1993). The QTL analysis also revealed that selection acting on individual salt tolerance QTLs was easily strong enough to counteract the homogenizing effect of gene flow, which is an important prerequisite in order for diploid hybrid speciation (i.e. sympatric/parapatric speciation) to occur (Lexer *et al.*, 2003c).

Most recently, research on the origin of stress adaptation in *Helianthus* has focused on two different aspects. One of them is the identification and study of the actual genes involved in adaptation, and the other one involves comparative genomic analyses across all three *Helianthus* hybrid species and their parents. In the case of salt tolerance in *H. paradoxus*, genetic mapping of candidate gene polymorphisms in field-grown hybrids allowed co-localization of fitness-related QTLs and genes (Lexer *et al.*, 2004), which revealed three salt tolerance candidate genes associated with fitness (survivorship) differences in the salt marsh 'hybrid' habitat. One of these, a gene coding for a calcium-dependent protein kinase (CDPK) isolated from stress-induced root tissue, mapped to a group of linked or pleiotropic salt tolerance QTLs expressed in the wild (Lexer *et al.*, 2004). Microarray-based expression studies of these and other candidates are currently underway, and preliminary results indicate

the involvement of additional candidate genes in salt stress adaptation (Zhao Lai & Loren Rieseberg, unpublished data). With respect to comparative genomic analyses, these studies revealed that the genomic composition of all three hybrid species could be predicted by a QTL analysis of synthetic early generation hybrids, hence providing convincing evidence that colonization of all three stressful 'hybrid' habitats (sand dunes, desert floors, salt marshes) had indeed been facilitated by hybridization (Rieseberg *et al.*, 2003).

Synthesis and conclusions for future studies

Many studies using a variety of different experimental approaches indicate that directional selection is an important cause of phenotypic diversification, and in many of these cases environmental stresses associated with divergent habitats or niches are likely to provide a target for selection. However, only few of these cases have been well characterized to the extent that the type of stress, traits involved in stress response, and genes controlling the traits have been identified. These include, for instance, heat shock proteins in *Drosophila* (Michalak *et al.*, 2001; Hoffmann *et al.*, 2003; Sorensen *et al.*, 2003) or plant defence genes in *Arabidopsis/Arabis* (Bishop *et al.*, 2000; Mitchell-Olds, 2001). For the vast majority of taxa/cases, this type of information is not available. In particular, for most taxa it still remains unknown if environmental stress is vital in the origin of ecological differences (as opposed to other factors such as mate choice in animals, pollinator preference in plants, or one of the many forms of resource partitioning between individuals), or to what extent environmental stress interacts with these factors. Also, it is important to distinguish between selective pressures generated by rare bouts of ecological stress vs. those created by adaptation to predictable and constant habitat conditions, whether they are harsh (in our perception) or not. Future studies should increasingly examine the role of environmental fluctuations, e.g. the proportion of evolutionary divergence that occurs as a consequence of extreme cold, heat or drought over different periods of time. Does selection generated by extreme environmental fluctuations create evolutionary trade-offs? Such trade-offs may occur because traits or trait combinations that are beneficial in one environment may not be so in another, i.e. the costs may outweigh the benefits in some environments. Long term selection studies (Grant & Grant, 2002) or molecular genetic studies of multi-generation responses to selection in the field (e.g. Ungerer *et al.*, 2003; Ungerer & Rieseberg, 2003) may help in addressing these topics.

We predict that, in organisms other than microbes, 'broad' natural hybrid zones maintained by environment-dependent selection will provide interesting venues for studying the role of environmental stress in adaptation and speciation (see Barton & Hewitt, 1985;

Harrison, 1990; Barton & Gale, 1993, for reviews, or Rieseberg et al., 1999; Vines et al., 2003; Johnston et al., 2001; Dodd & Afzal-Rafii, 2004; Lexer et al., 2004 for potential cases identified in the recent literature). Such natural interspecific populations allow researchers to circumvent the problem of generating experimental crosses in genetically less well tractable nonmodel organisms, they offer increased variance in phenotypic traits and fitness, and a well-developed framework exists for distinguishing between hybrid zones maintained by 'intrinsic' factors and those depending on ecology (Barton & Hewitt, 1985; Barton & Gale, 1993). A further advantage of hybrid zones is that differences in fitness can potentially be evaluated under field conditions over multiple generations, which is a prerequisite for assessing whether environmental stress can lead/has led to heritable evolutionary change in nature. Also, environmentally determined hybrid zones may provide a unique opportunity for comparing stress-adapted and non-adapted genotypes across environments, e.g. recombinant advanced generation hybrids carrying gene combinations from both parents may be adapted to stressful habitat patches within the hybrid zone while their parental genotypes may not be so. In this scenario, different responses would be induced in parental and hybrid genotypes. However, interpreting the evolutionary significance of divergent responses to new environments by hybrid and parental genotypes will require knowledge about the role of plasticity; phenotypic plasticity may also facilitate the occupation of new habitats, if trait changes in the new environment can be achieved through developmental changes induced by that environment (Levin, 2004).

There are a number of good reasons for picking out new study organisms for stress-related speciation research in plants. Their sessile nature and ease of crossing in many taxa renders plants ideal for addressing these questions through the combined use of selection experiments in natural environments, QTL mapping, population genetics and functional evolutionary genomics, as exemplified by the research on hybrid speciation in *Helianthus* reviewed here (Rieseberg *et al.*, 2002; Rosenthal *et al.*, 2002; Welch & Rieseberg, 2002; Lexer *et al.*, 2003b,c, 2004;), or by research on *Arabidopsis/Arabis* (Mitchell-Olds, 2001). Plants offer the added benefit that many biotic or abiotic stress responses have been well characterized on the molecular level because of their important role in agriculture (e.g. Knight, 2000; Hasegawa *et al.*, 2000a, b), and this knowledge is literally just waiting to be used for addressing the questions that remain – such as 'Is environmental stress a rare or frequent driver of speciation?'

Acknowledgments

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