Annals of Botany **108**: 777–787, 2011
doi:10.1093/aob/mcr180, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

REVIEW: PART OF A SPECIAL ISSUE ON SEXUAL PLANT REPRODUCTION

Plant sexual reproduction during climate change: gene function *in natura* studied by ecological and evolutionary systems biology

Kentaro K. Shimizu[1,*], Hiroshi Kudoh[2] and Masaki J. Kobayashi[1]

[1]*Institute of Plant Biology, University Research Priority Program in Systems Biology/Functional Genomics & Zurich-Basel Plant Science Center, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland and* [2]*Centre for Ecological Research, Kyoto University, Hirano 2-509-1, Otsu 520-2113, Japan*
** For correspondence. E-mail shimizu@botinst.uzh.ch*

Received: 30 November 2010 Returned for revision: 14 February 2011 Accepted: 18 May 2011 Published electronically: 17 August 2011

• *Background* It is essential to understand and predict the effects of changing environments on plants. This review focuses on the sexual reproduction of plants, as previous studies have suggested that this trait is particularly vulnerable to climate change, and because a number of ecologically and evolutionarily relevant genes have been identified.
• *Scope* It is proposed that studying gene functions in naturally fluctuating conditions, or gene functions *in natura*, is important to predict responses to changing environments. First, we discuss flowering time, an extensively studied example of phenotypic plasticity. The quantitative approaches of ecological and evolutionary systems biology have been used to analyse the expression of a key flowering gene, *FLC*, of *Arabidopsis halleri* in naturally fluctuating environments. Modelling showed that *FLC* acts as a quantitative tracer of the temperature over the preceding 6 weeks. The predictions of this model were verified experimentally, confirming its applicability to future climate changes. Second, the evolution of self-compatibility as exemplifying an evolutionary response is discussed. Evolutionary genomic and functional analyses have indicated that *A. thaliana* became self-compatible via a loss-of-function mutation in the male specificity gene, *SCR/SP11*. Self-compatibility evolved during glacial–interglacial cycles, suggesting its association with mate limitation during migration. Although the evolution of self-compatibility may confer short-term advantages, it is predicted to increase the risk of extinction in the long term because loss-of-function mutations are virtually irreversible.
• *Conclusions* Recent studies of *FLC* and *SCR* have identified gene functions *in natura* that are unlikely to be found in laboratory experiments. The significance of epigenetic changes and the study of non-model species with next-generation DNA sequencers is also discussed.

Key words: *Arabidopsis thaliana*, *Arabidopsis halleri*, climate change, *FLC*, *FLOWERING LOCUS C*, phenotypic plasticity, *SCR*, *S-LOCUS CYSTEINE-RICH PROTEIN*, evolution of selfing, predictive models, sexual reproduction, *SP11*, *S-LOCUS PROTEIN 11*, *SRK*, *S-RECEPTOR KINASE*, ecological and evolutionary systems biology.

INTRODUCTION: INTEGRATING EVOLUTION, ECOLOGY AND MOLECULAR BIOLOGY

The effects of changing environments on organisms are one of the foci of contemporary science (Intergovernmental Panel on Climate Change, 2007), and it is essential to understand and predict them. The negative effects of climate change on biodiversity and food production have been sources of concern (Intergovernmental Panel on Climate Change, 2002; Hedhly *et al.*, 2009). Such effects may depend largely on the responses of plants in terms of sexual reproduction, because plant reproductive success determines the levels of resources that support both biodiversity and the food supply, and because plant sexual reproduction is considered to be particularly vulnerable to the effects of global warming (Hedhly *et al.*, 2009; Eckert *et al.*, 2010). Hedhly *et al.* (2009) proposed that plant sexual reproduction under temperature stress could be altered both by phenotypic plasticity (non-genetic responses) and evolution (genetic responses). Phenotypic plasticity is defined as the capacity of a single genotype to produce a series of phenotypes in response to environmental changes. Evolutionary (or micro-evolutionary) responses imply changes in allele frequencies in populations over generations. Although many studies have focused on the effects of past and future climate changes on the ranges and abundance of species through migration, much less is known about phenotypic and evolutionary responses (Davis *et al.*, 2005; Gienapp *et al.*, 2008; Chown *et al.*, 2010).

Molecular genetic studies in laboratory conditions may not be adequate to study responses to changing environments, because laboratory environments can differ from natural habitats, characterized by large, stochastic fluctuations. The typical laboratory environment of *A. thaliana* includes adequate water, a constant warm temperature, and a lack of natural herbivores, which are not features of most of its natural habitats (Hoffmann, 2002). As a consequence, when mutants and natural accessions of *A. thaliana* are grown under field conditions, their observed phenotypic plasticity and fitness often differ from those observed under laboratory conditions

		Flowering-time genes including *FLC*	*SCR/SP11*
Function through interactions in ecosystems, or gene function *in natura*	Changing and fluctuating climates	Tracing long-term trend of temperature	Reproductive assurance in mate limitation
	Community (other species)	Avoidance of herbivory	spread by pollinator insects
	Population (the same species)	Synchronization for outcrossing	Mate choice (outcrossing vs. selfing)
Function in laboratories		Vernalization requirements	Male specificity of self-incompatibility

FIG. 1. Potential gene functions *in natura* or at multiple levels in ecosystems.

(Richards *et al.*, 2009; Wilczek *et al.*, 2009). Furthermore, mapping the loci of quantitative traits under natural conditions has identified a number of new loci, suggesting that mutations with no phenotypic effects in the laboratory could play specific roles in natural environments (Weinig *et al.*, 2002). In short, laboratory conditions provide a useful system, but this system may neglect the complexity of its interactions with its naturally fluctuating abiotic and biotic ecosystems. On the other hand, traditional quantitative genetic models may not have sufficient power to distinguish phenotypic plasticity and evolution because their assumptions, including heritability and genetic correlations, are often violated (Gienapp *et al.*, 2008). Gienapp *et al.* (2008) proposed that the use of genomic approaches to identify ecologically important variations would benefit the study of the biological responses to changing climates.

To understand and predict the biological responses to changing environments, we propose the importance of studying gene functions *in natura*, or gene functions in natural ecosystems (Fig. 1), analogous to the expression of 'immunology *in natura*' (Quintana-Murci *et al.*, 2007) in contrast to *in vivo* (in the living organism) and *in vitro* (in the test tube). It is essential to analyse gene functions or phenotypes at multiple levels in natural ecosystems, because genes *in natura* affect the interactions of an organism with individuals in a population of the same species, with other species in the community, or with fluctuating environments or climates (Fig. 1).

To study gene function *in natura*, it is obviously essential to integrate molecular biology, ecology and evolutionary biology. An established interdisciplinary field is evolutionary genomics (or evolutionary and ecological functional genomics) (Feder and Mitchell-Olds, 2003). Ecologically and evolutionarily relevant genes have been identified and studied using genomics to address evolutionary and ecological questions, including selective advantage in an ecological context and evolutionary timing during historic climatic fluctuations (Shimizu, 2002; Shimizu and Purugganan, 2005; Mitchell-Olds and Schmitt, 2006) (see the study of the evolution of self-compatibility explained below). Moreover, in this short review, we discuss that a broader integration incorporating systems biology and evolutionary genomics provides a powerful framework for predicting biological responses, in particular, plastic responses. Systems biology aims for biological understanding at the system level, and is characterized by large-scale quantitative data, modelling, networks and prediction (Kitano, 2002). So far, most systems biological research has been conducted in interdisciplinary collaborations, by applying mathematical and physical methods to molecular data. However, it should be emphasized that ecologists, evolutionary biologists and geneticists have been the pioneers of mathematical methods in biology (Begon *et al.*, 2006; Freeman and Herron, 2007; Benfey and Mitchell-Olds, 2008). To integrate the quantitative tools of ecology and evolutionary biology with those of molecular biology would establish a discipline of systems biology within an ecological and evolutionary context, called 'ecological and evolutionary systems biology' (Richards *et al.*, 2009). When combined with molecular biology, ecological data, such as long-term meteorological data, would provide useful tools to aid construction of a predictive model of gene functions *in natura*. A major aim of ecological and evolutionary systems biology would be to 'predict' both future and past responses of organisms to changing environments within the ecological and evolutionary contexts. We note that in climate studies, the terms 'projection' and 'forecasting' are preferred to 'prediction' because they imply a high degree of uncertainty. Here, the word 'prediction' is used in accordance with the custom of systems biology, but the uncertainty implied should not be forgotten.

To study the responses to changing environments, a model species like *A. thaliana* has an advantage because of the availability of a large amount of genetic and genomic data, but a single species cannot be adequate to study various ecologically and evolutionarily important traits. Close relatives of model species provide opportunities to investigate a wide range of ecological and evolutionary phenomena while exploiting the advantages a model species provides (Mitchell-Olds, 2001). This review focuses on a model plant, *A. thaliana*, and one of its closest relatives, *A. halleri*. *Arabidopsis halleri* can be stably transformed using an *Agrobacterium*-mediated technique (Hanikenne *et al.*, 2008), and has been used in studies of diverse ecological and evolutionary topics, including self-incompatibility (Bechsgaard *et al.*, 2006; Castric *et al.*, 2008; Tsuchimatsu *et al.*, 2010), perennial growth habit (Aikawa *et al.*, 2010), heavy metal tolerance (Roosens *et al.*, 2008; Hanikenne *et al.*, 2008), defence against herbivores (Shimizu, 2002; Kawagoe and Kudoh, 2010), polyploidization

(Shimizu-Inatsugi *et al.*, 2009; Schmickl *et al.*, 2010), phylogeography, and population structure (Van Rossum *et al.*, 2004; Meyer *et al.*, 2009; Heidel *et al.*, 2010).

Because the evolution, ecology and molecular biology of plant sexual reproduction have been extensively studied, sexual reproduction provides an ideal platform for interdisciplinary studies. To illustrate gene functions *in natura* and their potential application for the prediction of biological responses to changing climates, two aspects of our recent research are discussed: (1) as an example of phenotypic plasticity, a systems biological approach to construct a predictive model of the gene expression level of a key flowering gene *FLC* (*FLOWERING LOCUS C*) in naturally fluctuating environments; (2) the evolution of self-compatibility as an example of an evolutionary response. Genomic data has shown that self-compatibility in *A. thaliana* and other species evolved during recent glacial–interglacial cycles. It could be predicted that the evolution of self-compatibility may provide a short-term adaptation but entail a long-term risk of extinction.

PREDICTION OF PLASTIC RESPONSES BY MODELLING GENE EXPRESSION IN NATURAL ENVIRONMENTS

The flowering times of plants are highly plastic in response to diverse environmental factors, including temperature and day length. Recent climate change has shifted the flowering time of many plant species in various ecosystems, even though the flowering of plants in specific seasons is critical for plant reproductive success (Parmesan and Yohe, 2003; Korner and Basler, 2010; Wilczek *et al.*, 2010; Kobayashi and Shimizu, 2011). The molecular basis of flowering time control has been extensively studied in *A. thaliana* (Amasino, 2010; Fornara *et al.*, 2010). Recently, efforts to integrate ecological and molecular functional studies have been made to understand the function of flowering-time control in natural complex environments.

As one of the earliest attempts at an ecological and evolutionary systems biological approach, Wilczek *et al.* (2009) measured the flowering times in a series of *A. thaliana* strains, including mutants of the major flowering-time genes, in large-scale field experiments. The data were analysed using a photothermal model, which has long been used for the study of phenology (see the supplementary online material by Wilczek *et al.*, 2009). The model assumes that plants flower when a threshold number of photothermal units accumulate, with input from temperature and day length. By integrating the information derived from the flowering mutants with the molecular genetic network controlling flowering, the model can predict flowering time with a high degree of accuracy. Based on this model, Wilczek *et al.* (2010) also predicted future flowering times within a scenario of global warming.

Using another approach from systems biology, the expression of a key flowering-time gene in the vernalization pathway, *FLOWERING LOCUS C* (*FLC*), was monitored in natural habitats (Aikawa *et al.*, 2010). Because it has been noted that a critical problem in predictions that are based on mechanistic phenology models is the lack of any direct measurement of the internal state of the plant (Chuine *et al.*, 2003), these gene expression levels as representing such internal states were analysed. The question addressed was: what signals do plants receive that induce flowering in naturally fluctuating temperature regimes? Although it is well known that exposure to constant low temperatures for several weeks (vernalization) induces flowering in many plants, including *A. thaliana* (Michaels and Amasino, 1999; Sheldon *et al.*, 1999; Amasino, 2010), such stable conditions rarely exist in unpredictably fluctuating natural environments. For example, cold days could be followed by warm temperatures during autumn or early winter, but flowering in winter in response to such short temperature trends would be deleterious (Stinchcombe *et al.*, 2004). Therefore, the system of flowering-time control must detect the long-term trends in temperatures, even within the natural fluctuations of the environment, to ensure that they flower at the appropriate time.

FLC encodes a MADS-box transcription factor that functions as a repressor of the floral transition, and its expression is down-regulated by vernalization through epigenetic histone modifications (Michaels and Amasino, 1999; Sheldon *et al.*, 1999; Bastow *et al.*, 2004; Sung and Amasino, 2004) (http://www.arabidopsis.org/). *Arabidopsis halleri* subsp. *gemmifera* was used because its perennial habit allows the continuous observation of individuals for 2 years. Its *FLC* homologue (*AhgFLC*) repressed flowering when overexpressed in *A. thaliana*, indicating that its function as a floral repressor is conserved in *A. halleri* (Aikawa *et al.*, 2010). Tissues were collected from six individuals of a population of *A. halleri* subsp. *gemmifera* in central Japan, every week for 2 years, even during drought, flood and snow, and the expression levels of *FLC* were quantified with real-time PCR (Fig. 2). We note the critical advantage of using sessile organisms in this type of study, as it is generally feasible to repeatedly locate individual plants and to harvest their tissues for molecular studies. Seasonal changes in the *AhgFLC* expression levels occurred slowly, reflecting the temperature trend of approx. 6 preceding weeks. A time series analysis was conducted to construct a quantitative model of the time course of the expression of this gene. The chilling accumulation model, which has been used in phenological research in ecology (Chuine *et al.*, 2003), was used to analyse expression levels with the hourly ground temperature recorded using a data logger. The analysis showed that up to 83 % of the variation across 576 expression data points was explained solely by the temperature over the preceding 6 weeks (Aikawa *et al.*, 2010). The predictions of the model were tested further by exposing plants to controlled laboratory conditions, and the predictions of the model accorded well with the expression levels of *AhgFLC* in these artificial transplantation experiments.

From the viewpoint of molecular genetics, *FLC* is described as encoding a floral repressor, the expression of which is down-regulated by long exposure to low temperatures. If the environments in the natural habitat are taken into consideration, the *FLC* expression level can be considered a quantitative tracer of the temperature over the preceding 6 weeks (Fig. 1). Using the terminology of electronics or systems

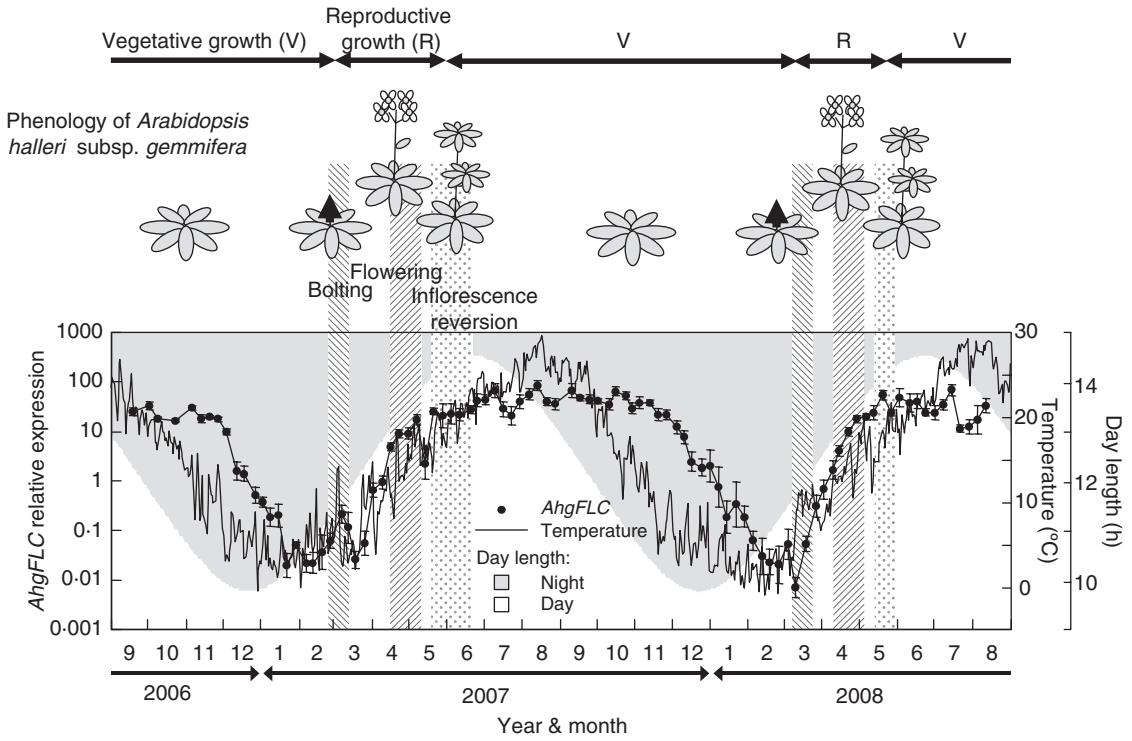

FIG. 2. Phenology of *Arabidopsis halleri* subsp. *gemmifera* and the relative expression levels of *AhgFLC*.

biology, the function of *FLC in natura* is a low-pass filter to extract a long-term trend in temperature by neglecting short-term fluctuations (Bennett *et al.*, 2008; Aikawa *et al.*, 2010).

The accuracy of the models developed with the two approaches described above (Wilczek *et al.*, 2009, 2010; Aikawa *et al.*, 2010) indicates that the molecular basis of flowering time regulation can contribute to the prediction of plant responses to changing climates. To understand fully the functions of flowering-time control genes *in natura*, processes at the population and community levels as well as other environmental signals should be also incorporated (Fig. 1). For example, selection for early flowering to avoid floral herbivory has been detected in the same population of *A. halleri* in which *AhgFLC* expression was studied (Kawagoe and Kudoh, 2010). In terms of the interaction of a plant with other individuals within the population, outcrossing species, like *A. halleri*, should flower synchronously with conspecifics to maximize the potential for reproductive success (Satake, 2004). A future question to be addressed is how interactions at multiple levels affect the plastic and evolutionary responses of the flowering-time genes to climate changes.

LEARNING FROM THE PAST FOR EVOLUTIONARY PREDICTION: SELF-COMPATIBILITY OF *ARABIDOPSIS THALIANA*

Another challenge is to predict evolutionary responses to global climate change by learning from the evolution that has occurred during past climate changes. If all evolutionary adaptations were only required on a time scale of millions of years, migration would be the main response to climate change. However, Davis *et al.* (2005) emphasized that adaptive microevolution can occur much more rapidly than this in response to the climatic changes induced by glacial cycles, with periods of 100 000 years, combined with variations on millennial, centennial and even decadal time scales, and further evidence of the rapid evolutionary dynamics affecting ecological traits has recently been reported (Schoener, 2011). At the Evolution 2010 meeting in Oregon in June 2010, a symposium on 'Moving towards a science of evolutionary prediction' suggested that the next challenge will be to improve the accuracy of evolutionary prediction (http://www.evolutionsociety.org/SSE2010), although the prediction of evolution is still difficult. Therefore, we propose to investigate relatively simple cases, focusing on loss-of-function mutations.

Here, the evolutionary loss of self-incompatibility is discussed, because it is considered one of the most frequent evolutionary shifts in angiosperms (Stebbins, 1974; Charlesworth, 2006), and because loss-of-function mutations have been shown to underlie this loss (Tsuchimatsu *et al.*, 2010). First, the molecular basis of self-incompatibility in the Brassicaceae and recent studies of the evolution of self-compatibility in *A. thaliana* (Shimizu *et al.*, 2008; Tsuchimatsu *et al.*, 2010) will be summarized briefly. Then the estimated time of the evolution of self-compatibility will be described using molecular data which suggest its origins during recent glacial–interglacial cycles. Based on these points, how molecular data, focusing on gene function *in natura*, can contribute to evolutionary prediction is discussed, and the suggestion is made that the evolution of self-compatibility entails the long-term risk of extinction, despite the short-term adaptation it has afforded.

Molecular basis of self-incompatibility and self-compatibility

Many Brassicaceae species, including *A. halleri*, *A. lyrata* and *Brassica* species, have a sporophytic self-incompatible system to avoid self-fertilization. The incompatibility response is based on the female specificity gene *S-RECEPTOR KINASE* (*SRK*) and the male specificity gene *S-LOCUS CYSTEINE-RICH PROTEIN* (*SCR*; also denoted *S-LOCUS PROTEIN 11*, *SP11*), and both are encoded at the *S*-locus (Takayama and Isogai, 2005). *SRK* encodes a transmembrane serine/threonine receptor kinase expressed in the stigma, whereas *SCR* encodes a small cysteine-rich protein ligand present on the pollen surface (Suzuki *et al.*, 1999; Schopfer *et al.*, 1999; Takasaki *et al.*, 2000; Takayama *et al.*, 2000). The specificity of self-recognition is conferred by *S*-haplogroups (*S*-haplotypes or *S*-alleles), because the SCR and SRK proteins derived from the same *S*-haplogroups bind one another and inhibit the growth of the pollen tubes (Takayama *et al.*, 2001; Kachroo *et al.*, 2001). Tens of *S*-haplogroups with high sequence divergence are maintained within populations by frequency-dependent selection, because they confer a minority advantage (Castric *et al.*, 2008; Llaurens *et al.*, 2008).

Arabidopsis thaliana is self-compatible and a predominant selfer (selfing rate of 97 % or higher) (Abbott and Gomes, 1989; Platt *et al.*, 2010). The genetic basis of self-compatibility has been extensively studied, and many mutations and deletions at the *S*-locus have been reported (Kusaba *et al.*, 2001; Nasrallah *et al.*, 2002; Tang *et al.*, 2007; Sherman-Broyles *et al.*, 2007; Liu *et al.*, 2007; Shimizu *et al.*, 2008; Mable, 2008; Boggs *et al.*, 2009). However, many authors have pointed out that it is difficult to identify the primary inactivating mutation because other genes experience secondary decay once the self-incompatibility function is lost (Igic *et al.*, 2008; Busch and Schoen, 2008; Boggs *et al.*, 2009). The *S*-locus at the population level has been sequenced and genotyped (Shimizu *et al.*, 2008; Tsuchimatsu *et al.*, 2010), and a disruptive 213-bp inversion in the male *SCR-A* gene of haplogroup A or its deletion derivative found in 95 % of European *A. thaliana* accessions. This pattern contrasts with the population structure, which is thought to have been shaped by glacial–interglacial cycles, suggesting that the inversion mutation conferring self-compatibility spread by natural selection. In contrast to *SCR*, many accessions express the full-length *SRK-A* gene. These accessions of *A. thaliana* showed a self-incompatibility response at the stigma when the pollen grains of *A. halleri* of haplogroup A were crossed. This evolution has also been successfully reversed experimentally, i.e. when the 213-bp inversion of the *SCR* gene was restored, the transgenic plants became self-incompatible. These results show that many accessions of *A. thaliana* are still self-incompatible in terms of the female function, and that a loss-of-function mutation in the male specificity gene was responsible for the evolution of self-compatibility.

The evolution of self-compatibility during glacial cycles

The timing of evolution of selfing or self-compatibility in *A. thaliana* has been studied by many researchers, and is somewhat contentious. On the one hand, an origin of the selfing estimated to be approximately one million years before present was suggested by a simulation of genome-wide linkage disequilibrium (Tang *et al.*, 2007), which provides ample time for the evolution of 'the selfing syndrome' (small petals/flowers and a reduction in the number of pollen grains) (Barrett, 2002). Conversely, Bechsgaard *et al.* (2006) estimated the time of the evolution of self-compatibility using a molecular evolutionary approach, and developed a method based on the $d_N : d_S$ ratio (also called the '$K_a : K_s$ ratio', the ratio of the rate of non-synonymous substitutions per site to the rate of synonymous substitutions per site). The $d_N : d_S$ ratios of functional genes are much <1 because of the functional constraints on the encoded proteins. However, once a gene becomes a pseudogene, the non-synonymous positions evolve neutrally, so the $d_N : d_S$ ratio will gradually approach a value of 1. Interestingly, the $d_N : d_S$ ratio of *SRK* in the *A. thaliana* lineage shows no pseudogenization signature. The calculations of Bechsgaard *et al.* showed that *A. thaliana* has been self-incompatible for at least 91.7 % of the time since it diverged from *A. lyrata* and *A. halleri* (Bechsgaard *et al.*, 2006). To translate this value into years, it is necessary to estimate the time at which *A. thaliana* diverged from the other *Arabidopsis* species, which is currently under discussion. If a commonly used divergence time of five million years (Koch *et al.*, 2000) is assumed, the loss of self-incompatibility is estimated to have occurred more recently than 0·413 million years ago (mya) (Bechsgaard *et al.*, 2006). Recently, Beilstein *et al.* (2010) noted that the estimate by Koch *et al.* (2000) was based on a misunderstanding of the fossil literature (Beilstein *et al.*, 2010). More recent reports have proposed a much earlier split at 13·0 mya, with a 95 % confidence interval of 8·0–17·9 mya, according to a calibration based on multiple fossil records by Beilstein *et al.* (2010); or 8·7 $\pm$ 1·0 mya or 17·9 $\pm$ 4·8 mya based on mutation accumulation lines, proposed by Ossowski *et al.* (2010). Using these values, self-compatibility originated 0–0·64 mya (assuming a minimum divergence estimate of 7·7 mya) or 0–1·88 mya (assuming a maximum divergence estimate of 22·7 mya). The time ranges are well within the Quaternary, which is characterized by 41-ky and 100-ky cycles of glacial–interglacial periods. Therefore, these estimates indicate that the evolution of self-compatibility probably occurred during a glacial–interglacial cycle, providing an example of an evolutionary response to climate change.

The present study supports the recent origin of self-compatibility proposed by the molecular evolutionary analysis in two respects (Tsuchimatsu *et al.*, 2010). First, all the genes necessary for the self-recognition and rejection response, except *SCR*, still retain functional alleles in many accessions of *A. thaliana*. If self-incompatibility were lost a long time ago, the other genetic components would have been degraded, although pleiotropy could have slowed this process (Tantikanjana *et al.*, 2009). Second, the pattern of polymorphisms at the *S*-locus has deviated from the genome-wide pattern, which is thought to have been shaped by range expansions from multiple refugia during glacial–interglacial cycles (Sharbel *et al.*, 2000; Nordborg *et al.*, 2005; Schmid *et al.*, 2006; Beck *et al.*, 2008; Francois *et al.*, 2008).

In addition to the molecular functional and evolutionary genomic studies of *A. thaliana*, phylogenetic studies of the

family Solanaceae have shown that self-compatible species are short lived because of their higher extinction rates (Goldberg *et al.*, 2010), and thus supports the hypothesis for recent origin of self-compatibility. Population genetic studies have also suggested a recent origin of self-compatibility in *Capsella rubella*, *A. kamchatica*, and North American *A. lyrata* (Shimizu *et al.*, 2005; Sugisaka and Kudoh, 2008; Shimizu-Inatsugi *et al.*, 2009; Foxe *et al.*, 2009, 2010; Guo *et al.*, 2009; Hoebe *et al.*, 2009). Taken together, the evolution of self-compatibility during recent glacial–interglacial cycles appears to be a broad, general pattern. It should be noted that time estimates based on population genetics also depend on molecular clock calibration, as described above, and also require a number of assumptions to be made about the population demography. A recent large-scale polymorphism study in *A. thaliana* showed a continuous gradient of variations along eastern–western Europe (Platt *et al.*, 2010), suggesting that methods are still required to estimate time and to test neutrality within a continuous population structure.

The recent origins of self-compatibility also suggest that the 'selfing syndrome' (Barrett, 2002) evolves rapidly. Although the data presented above demonstrates that the complete loss of self-compatibility occurred recently, this does not necessarily mean that the ancestral *A. thaliana* was highly outcrossing, with strong self-incompatibility. In many wild species, partial self-compatibility results in mixed mating systems (Goodwillie *et al.*, 2005). Interestingly, it has been observed that self-incompatibility is weakened in the later stages of flower development in many *A. thaliana* accessions but not in other accessions (Tsuchimatsu *et al.*, 2010). Such partial self-compatibility would provide opportunities for reproductive assurance by selfing if no outcrossing pollen is available. It remains to be clarified whether leaky self-incompatibility is the ancestral state of *A. thaliana*, or represents secondary decay after the evolution of self-compatibility.

Using gene function in natura *for evolutionary prediction*

Laboratory studies have demonstrated that *SCR* encodes a protein ligand of SRK. At the population level, functional *SCR* and *SRK* genes enforce outcrossing (Fig. 1). Moreover, the studies described above suggested the loss-of-function mutation of *SCR* contributed to adaptation in a changing climate. Charles Darwin (1876) proposed a hypothesis for reproductive assurance, which states that selfing can be advantageous when mates or pollinators are scarce. The evolution of self-compatibility in many species including *A. thaliana* appears to have occurred during recent glacial–interglacial cycles. Migration, such as that occurring during range expansion after glacial periods, would be accompanied by a paucity of mates (Baker, 1955). Thus, the origin of self-compatibility glacial–interglacial cycles, together with the spread of the self-compatible mutation at the *S*-locus, suggests that the loss-of-function mutation of *SCR* could have been responsible for mating system shift thus allowing reproductive assurance during glacial–interglacial cycles.

Furthermore, the loss-of-function mutation of *SCR* in *A. thaliana* illustrates three ways in which integrated studies using molecular data contribute to our understanding and ability to predict evolutionary and ecological phenomena. First, the independent origins of self-compatibility were revealed in the studies of the self-compatibility mutations. The self-compatibility of accessions with haplogroup B, distributed on offshore African islands, evolved independently, because they do not contain the 213-bp inversion in *SCR-A* (Tang *et al.*, 2007; Shimizu *et al.*, 2008; Boggs *et al.*, 2009). It is also possible that individuals with haplogroup C have yet another independent self-compatible mutation. All natural plants of *A. thaliana* reported so far are self-compatible, i.e. the self-compatibility phenotype is fixed in *A. thaliana*, so it is not possible to identify the parallel evolution of self-compatibility from the self-compatible phenotype alone. A study at the species level suggested that the evolution of self-compatibility is one of the most frequent evolutionary transitions in angiosperms (Stebbins, 1974), but it must be even more frequent than has been previously thought because a single species could include parallel transitions. A growing number of studies have shown the parallel evolution of various phenotypes in many species, particularly those caused by loss-of-function mutations (Hoekstra and Coyne, 2007; Shimizu and Purugganan, 2005). Therefore, evolutionary studies based on phenotypic frequencies could overlook the complexity of their evolution. Second, because of the prevalence of loss-of-function mutations, the direction of evolution is often asymmetric or even unidirectional, which is consistent with Dollo's law that states character loss is irreversible (Zufall and Rausher, 2004). Although the evolutionary transition from self-incompatibility to self-compatibility has occurred independently many times, evolution in the opposite direction is considered to be extremely rare (Stebbins, 1974; Igic *et al.*, 2006; Goldberg *et al.*, 2010). Evolutionary ecological models that assume symmetric and small-effect mutations have shown that predominant selfing is a stable state among the mating systems (Lande and Schemske, 1985), suggesting that evolution towards predominant selfing is asymmetrically preferred by selection. When we focus on mutation rather than on selection, a simpler explanation is possible, because loss-of-function mutations (nonsense mutations, splicing mutations, frame-shift mutations, etc.) are expected to occur much more frequently than back mutations. Although the transgenic experiments successfully reversed the 213-bp mutation in *SCR* (Tsuchimatsu *et al.*, 2010), the chance of this exact mutation occurring under natural conditions is extremely small. Moreover, many self-incompatibility haplogroups are required to maintain efficient outcrossing. Whereas >30 *S*-haplogroups have been identified in both self-incompatible *A. lyrata* and *A. halleri* (Castric *et al.*, 2008), only three haplogroups are reported in *A. thaliana* (Shimizu *et al.*, 2008). The evolution of new haplogroups would require multiple mutations to allow the co-evolution of the male and female specificity genes, although little is known about the molecular basis of their evolution (Newbigin and Uyenoyama, 2005). We would also like to note that rapid reverse evolution may be possible if the alleles are still segregating (Kitano *et al.*, 2008). Third, future evolvability would be restricted by the nature of the loss-of-function mutations. Once self-incompatibility is lost, it is unlikely to be regained, because the evolution of a new self-incompatibility system is thought to have occurred only several times in the history of the angiosperms (de

Nettancourt, 2000). Furthermore, although empirical data are limited, it is generally considered that predominantly selfing species may not respond to environmental changes because their genetic diversity is reduced (Takebayashi and Morrell, 2001; Goldberg *et al.*, 2010).

What can be predicted about the evolution of self-compatibility from the integrated studies of molecular biology, ecology and evolution? For example, climate models indicate that global warming will have a major impact on alpine flora, including the fragmentation of habitat, because alpine environments will be subjected to dramatic and rapid environmental changes (Parmesan, 2006; Intergovernmental Panel on Climate Change, 2007). When habitats are fragmented, the evolution of predominant selfing by the loss of self-incompatibility may assure the reproduction of plants by conferring a short-term advantage. However, it is reported that self-compatible lineages suffer from extinction (Goldberg *et al.*, 2010). Thus, it is predicted that these plants will not regain their self-incompatibility in the short term, even if the environment is restored, and will therefore be subject to higher extinction rates in the longer term. In short, rapid adaptive evolution may confer short-term advantages, but entail long-term extinctions.

The mutation of *SCR* was relatively easy to analyse because its loss of function has a major effect on self-compatibility. However, ecologically and evolutionarily relevant traits may be affected by a large number of small-effect mutations. Analysing these mutations to identify general patterns will require a quantitative and network approach, using genome-wide surveys of wild organisms.

CONCLUSIONS AND PERSPECTIVES

Gene functions in natura

To predict biological responses to changing environments, it is important to analyse gene functions or phenotypes in naturally fluctuating environments, which are often very different from laboratory conditions (Fig. 1). Studies of *FLC* and *SCR* have examined gene functions *in natura*, demonstrating that genes (or alleles) function at multiple levels in ecosystems in the context of various biotic and abiotic interactions (Fig. 1). In the laboratory, FLC functions as a floral suppressor, unless the plant is exposed to low temperatures for several weeks. However, an expression study in naturally fluctuating environments revealed that *FLC* acts as a tracer of temperature trends in the preceding several weeks (Aikawa *et al.*, 2010). Such an extended view of gene function is essential for the prediction of plant responses to changing climates. Similarly, we have described that fixation of a non-functional *SCR* allele could have changed the mating system in plant populations, thereby contributing to reproductive assurance during migrations within glacial cycles. In addition, the interaction with pollinators may be also important for the evolution of self-compatibility. Theoretical analyses have predicted that when selfing is favoured, the frequencies of the mutations that disable the male component of self-incompatibility will increase more than the frequencies of the mutations that disable the female components (Uyenoyama *et al.*, 2001; Busch and Schoen, 2008). This is because male mutations are propagated through both pollen and seed, whereas female mutations are only propagated through seed. Crossing with *A. halleri* and transgenic data have shown that an *SCR* mutation is primarily responsible for self-compatibility according to this prediction. Thus, mutations in the male specificity components suggest that pollinator insects might have assisted the spread of the *SCR* allele (Tsuchimatsu *et al.*, 2010) (Fig. 1). In short, genes can affect the interactions of an organism with individuals in a population of the same species, with other species in the community, and with fluctuating environments or climates (Fig. 1). Phenotypes within ecosystems are often called ‘extended phenotypes’ (Whitham *et al.*, 2006).

The limitations of laboratory experiments are evident in a classic example of the human glucose-6-phosphate dehydrogenase gene (*G6PD*) (Freeman and Herron, 2007). The *G6PD* gene is usually considered a ‘housekeeping’ gene, encoding an enzyme in the pentose phosphate pathway. However, deficiency alleles are frequently found in tropical human populations, and these confer resistance to severe malaria. It is reasonable to infer that a function of a deficient *G6PD* allele is to confer malaria resistance, although it is implausible that a laboratory experiment alone would identify this function in the interaction between humans and other species in an ecosystem.

We would like to add that plasticity, evolution and migration are not independent. Different genotypes show different phenotypic plasticity, so plasticity itself must evolve (Gienapp *et al.*, 2008). Indeed, the association between the plastic response of flowering and *FLC* polymorphisms was detected in *A. thaliana* (Caicedo *et al.*, 2004). Migration can cause mate limitation and the evolution of self-compatibility (Tsuchimatsu *et al.*, 2010), and then self-compatibility can, in turn, facilitate migration. Thus, it is important to integrate ecological and evolutionary information to understand the responses of a species to changing climate.

Epigenetics and next-generation DNA sequencing

Epigenetic changes may have played a significant role in evolution and ecology (Kalisz and Purugganan, 2004; Bossdorf *et al.*, 2008), and more specifically, in organisms’ responses to changing climates, but little is currently known. Several studies have started to show heritable epigenetic variations within and among species (Fujimoto *et al.*, 2008; Johannes *et al.*, 2009). The transcriptional activity of *FLC* is mediated by epigenetic histone modifications on the *FLC* chromatin. During vernalization, the methylation at histone H3 Lys9 and histone H3 Lys27 (H3K27) on the *FLC* chromatin increases, resulting in the repression of *FLC* transcription (Bastow *et al.*, 2004; Sung and Amasino, 2004). In the annual species *A. thaliana*, the repressed status of *FLC* is stable, even when the plants are returned to warm temperatures. In contrast, in the perennial species *Arabis alpina*, the repressed status of *PEP1* (*FLC* homologue) is transient (Wang *et al.*, 2009). The H3K27 methylation levels on the *PEP1* chromatin increase in *Arabis alpina* during vernalization, corresponding to the expression pattern of *PEP1*, but they then decrease when the plants experience warmer temperatures. These data suggest that epigenetic regulation underlies the differences between the annual and perennial

growth forms. Interestingly, yeasts detect long-term trends of nutrient availability through a slow chemical reaction in a metabolic network (Bennett *et al.*, 2008), and it is conceivable that the slow epigenetic regulation of *FLC* underlies the plants' detection of seasonal trends.

In addition to the effect of prolonged low temperature in flowering time regulation discussed above, *A. thaliana* shows plasticity of flowering time in response to temperatures in the range of non-stress conditions (16–27 °C), called ambient temperatures. It is reported that an increase in growth temperature causes early flowering (Westerman and Lawrence, 1970; Blázquez *et al.*, 2003; Lempe *et al.*, 2005). Recently, nucleosomes containing histone variant H2A.Z have been found to mediate the thermosensory response (Kumar and Wigge, 2010). H2A.Z-containing nucleosomes wrapped DNA more tightly, and then transcription of one of the key flowering activators, *FLOWERING LOCUS T*, was changed. These data imply the link between epigenetic regulation through nucleosome assembly and the plasticity of temperature-dependent flowering time. It would be one of challenges to study the significance of epigenetic changes in response to global warming.

The dominance relationship of *SCR/SP11* haplogroups is also regulated epigenetically by the DNA methylation induced by a small RNA (Shiba *et al.*, 2006; Tarutani *et al.*, 2010). The sporophytic self-incompatibility system in the family Brassicaceae is characterized by dominance relationships between haplogroups, in which heterozygotes often show only one of their two *S*-specificities (Hatakeyama *et al.*, 1998). The most recessive alleles exhibit higher frequencies in outcrossing populations (33 % in a population of *A. lyrata*; Mable *et al.*, 2003), whereas the dominant alleles show much lower frequencies. Haplogroups in the same dominance class also tend to cluster on phylogenetic trees (Prigoda *et al.*, 2005). It is noteworthy that haplogroups A, B and C of *A. thaliana* (Shimizu *et al.*, 2008) belong to clades of dominant classes. In particular, haplogroup A (AhS04 of *A. halleri*), from which the self-compatible mutation spread in *A. thaliana* (Tsuchimatsu *et al.*, 2010), has been shown experimentally to be a dominant allele in *A. halleri* (Llaurens *et al.*, 2008). If a loss-of-function mutant of *SCR* still retains its dominance relationship and functions as a dominant self-compatible mutation by repressing the expression of another specificity, this allele is expected to spread more rapidly than a recessive self-compatible mutation. Interestingly, studies of *Brassica* have shown that the *S*-locus harbours a separate gene (*Smi*) responsible for the dominance effect, which encodes a small RNA that represses the expression of allelic *SCR* (Shiba *et al.*, 2006; Tarutani *et al.*, 2010). Therefore, the loss-of-function mutation in *SCR* may yield a dominant self-compatibility allele, which would contribute to the rapid spread of self-compatibility. Furthermore, studies of *Brassica* have also suggested that self-compatible alleles that are dominant can confer self-compatibility on polyploid species (Okamoto *et al.*, 2007). It remains to be demonstrated whether *Smi* is functional in *Arabidopsis*.

Such recent advances in epigenetic studies in the laboratory indicate that both epigenetic and genetic regulation must be considered together to understand phenotypic plasticity and evolution. To quantify the contribution of epigenetic states in natural environments is another future challenge.

In this review, we have explained that utilizing *A. halleri*, a close relative of a model plant *A. thaliana*, enabled the study of perennial habit and self-incompatibility, which is not found in *A. thaliana*. A future challenge for ecological and evolutionary systems biology will be to apply the methods developed for model species to other species (Karrenberg and Widmer, 2008), in particular to keystone species, which affect many other organisms in an ecosystem. Next-generation DNA sequencers, initially developed for medical purposes, are now revolutionizing ecological and evolutionary biology (Benfey and Mitchell-Olds, 2008; Kobayashi and Shimizu, 2011). These sequencers will provide genome-wide data on 'non-model' species, including keystone plant and animal species, even when little genomic information is already available. For example, sequencing cDNA allows genome-wide expression patterns to be investigated, and higher resolution can be achieved by combining this technology with customized microarrays (Toth *et al.*, 2007; Bellin *et al.*, 2009; Leakey *et al.*, 2009). These techniques will facilitate the study of more non-model species, to establish general patterns and to predict their phenotypic plasticity and evolutionary responses to coming climate changes.

ACKNOWLEDGEMENTS

We thank Vincent Castric, Akiko Satake, Rie Shimizu-Inatsugi, Andrew Tedder, Takashi Tsuchimatsu, Xavier Vekemans, Masao Watanabe and anonymous reviewers for their valuable suggestions. This work was supported by the University Research Priority Program in Systems Biology/Functional Genomics of the University of Zurich, the Swiss National Science Foundation (SNF), SystemsX.ch and Grants-in-Aid for Special Research on Priority Areas (119043010) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, the Global COE Program A06 of Kyoto University and the NEXT Program (GS013).

LITERATURE CITED

Abbott RJ, Gomes MF. 1989. Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. *Heredity* **62**: 411–418.

Aikawa S, Kobayashi MJ, Satake A, Shimizu KK, Kudoh H. 2010. Robust control of the seasonal expression of the *Arabidopsis FLC* gene in a fluctuating environment. *Proceedings of the National Academy of Sciences of the USA* **107**: 11632–11637.

Amasino R. 2010. Seasonal and developmental timing of flowering. *The Plant Journal* **61**: 1001–1013.

Baker HG. 1955. Self compatibility and establishment after long distance dispersal. *Evolution* **9**: 347–349.

Barrett SC. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**: 274–284.

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of *FLC* by histone methylation. *Nature* **427**: 164–167.

Bechsgaard JS, Castric V, Vekemans X, Schierup MH. 2006. The transition to self-compatibility in *Arabidopsis thaliana* and evolution within S-haplotypes over 10 myr. *Molecular Biology and Evolution* **23**: 1741–1750.

Beck JB, Schmuths H, Schaal BA. 2008. Native range genetic variation in *Arabidopsis thaliana* is strongly geographically structured and reflects Pleistocene glacial dynamics. *Molecular Ecology* **17**: 902–915.

Begon M, Townsend CR, Harper JL. 2006. *Ecology: from individuals to ecosystems.* Malden, MA: Blackwell.

Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S. 2010. Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* **107**: 18724–18728.

Bellin D, Ferrarini A, Chimento A, *et al*. 2009. Combining next-generation pyrosequencing with microarray for large scale expression analysis in non-model species. *BMC Genomics*, **10**: 555. doi:10.1186/1471-2164-10-555.

Benfey PN, Mitchell-Olds T. 2008. From genotype to phenotype: systems biology meets natural variation. *Science* **320**: 495–497.

Bennett MR, Pang WL, Ostroff NA, *et al*. 2008. Metabolic gene regulation in a dynamically changing environment. *Nature* **454**: 1119–1122.

Blazquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* **33**: 168–171.

Boggs NA, Nasrallah JB, Nasrallah ME. 2009. Independent *S*-locus mutations caused self-fertility in *Arabidopsis thaliana*. *PLoS Genetics* **5**: e1000426. doi:10.1371/journal.pgen.1000426.

Bossdorf O, Richards CL, Pigliucci M. 2008. Epigenetics for ecologists. *Ecology Letters* **11**: 106–115.

Busch JW, Schoen DJ. 2008. The evolution of self-incompatibility when mates are limiting. *Trends in Plant Science* **13**: 128–136.

Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD. 2004. Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences of the USA* **101**: 15670–15675.

Castric V, Bechsgaard J, Schierup MH, Vekemans X. 2008. Repeated adaptive introgression at a gene under multiallelic balancing selection. *PLoS Genetics* **4**: e1000168. doi:10.1371/journal.pgen.1000168.

Charlesworth D. 2006. Evolution of plant breeding systems. *Current Biology* **16**: R726–R735.

Chown SL, Hoffmann AA, Kristensen TN, Angilletta MJ, Stenseth NC, Pertoldi C. 2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* **43**: 3–15.

Chuine I, Kramer K, Hanninen H. 2003. Plant development models. In: Schwartz MD. ed. *Phenology: an integrative environmental science*. Dordrecht: Kluwer, 217–235.

Darwin C. 1876.*The effects of cross and self fertilization in the vegetable kingdom*. London: John Murray.

Davis MB, Shaw RG, Etterson JR. 2005. Evolutionary responses to changing climate. *Ecology* **86**: 1704–1714.

Eckert CG, Kalisz S, Geber MA, *et al*. 2010. Plant mating systems in a changing world. *Trends in Ecology & Evolution* **25**: 35–43.

Feder ME, Mitchell-Olds T. 2003. Evolutionary and ecological functional genomics. *Nature Reviews Genetics* **4**: 651–657.

Fornara F, de Montaigu A, Coupland G. 2010. SnapShot: control of flowering in *Arabidopsis*. *Cell* **141**: 550.

Foxe JP, Slotte T, Stahl EA, Neuffer B, Hurka H, Wright SI. 2009. Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences of the USA* **106**: 5241–5245.

Foxe JP, Stift M, Tedder A, Haudry A, Wright SI, Mable BK. 2010. Reconstructing origins of loss of self-incompatibility and selfing in North American *Arabidopsis lyrata*: a population genetic context. *Evolution* **64**: 3495–3510.

Francois O, Blum MG, Jakobsson M, Rosenberg NA. 2008. Demographic history of European populations of *Arabidopsis thaliana*. *PLoS Genetics* **4**: e1000075. doi:10.1371/journal.pgen.1000075.

Freeman S, Herron JC. 2007.*Evolutionary analysis*. Upper Saddle River, NJ: Pearson.

Fujimoto R, Kinoshita Y, Kawabe A, *et al*. 2008. Evolution and control of imprinted *FWA* genes in the genus *Arabidopsis*. *PLoS Genetics* **4**: e1000048. doi:10.1371/journal.pgen.1000048.

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merila J. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* **17**: 167–178.

Goldberg EE, Kohn JR, Lande R, Robertson KA, Smith SA, Igic B. 2010. Species selection maintains self-incompatibility. *Science* **330**: 493–495.

Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* **36**: 47–79.

Guo YL, Bechsgaard JS, Slotte T, *et al*. 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the USA* **106**: 5246–5251.

Hanikenne M, Talke IN, Haydon MJ, *et al*. 2008. Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of *HMA4*. *Nature* **453**: 391–395.

Hatakeyama K, Watanabe M, Takasaki T, Ojima K, Hinata K. 1998. Dominance relationships between *S*-alleles in self-incompatible *Brassica campestris* L. *Heredity* **80**: 241–247.

Hedhly A, Hormaza JI, Herrero M. 2009. Global warming and sexual plant reproduction. *Trends in Plant Science* **14**: 30–36.

Heidel AJ, Ramos-Onsins SE, Wang WK, Chiang TY, Mitchell-Olds T. 2010. Population history in *Arabidopsis halleri* using multilocus analysis. *Molecular Ecology* **19**: 3364–3379.

Hoebe PN, Stift M, Tedder A, Mable BK. 2009. Multiple losses of self-incompatibility in North American *Arabidopsis lyrata*? Phylogeographic context and population genetic consequences. *Molecular Ecology* **18**: 4924–4939.

Hoekstra HE, Coyne JA. 2007. The locus of evolution: evo devo and the genetics of adaptation. *Evolution* **61**: 995–1016.

Hoffmann MH. 2002. Biogeography of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae). *Journal of Biogeography* **29**: 125–134.

Igic B, Bohs L, Kohn JR. 2006. Ancient polymorphism reveals unidirectional breeding system shifts. *Proceedings of the National Academy of Sciences of the USA* **103**: 1359–1363.

Igic B, Lande R, Kohn JR. 2008. Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* **169**: 93–104.

Intergovernmental Panel on Climate Change. 2002. *Climate change and biodiversity*. IPCC Technical Paper V., Gitay H. *et al.* eds. Geneva, Switzerland: IPCC.

Intergovernmental Panel on Climate Change. 2007. *Climate change. 2007 synthesis report*. Geneva, Switzerland: IPCC.

Johannes F, Porcher E, Teixeira FK *et al*. 2009. Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genetics* **5**: e1000530. doi:10.1371/journal.pgen.1000530.

Kachroo A, Schopfer CR, Nasrallah ME, Nasrallah JB. 2001. Allele-specific receptor–ligand interactions in *Brassica* self-incompatibility. *Science* **293**: 1824–1826.

Kalisz S, Purugganan MD. 2004. Epialleles via DNA methylation: consequences for plant evolution. *Trends in Ecology & Evolution* **19**: 309–314.

Karrenberg S, Widmer A. 2008. Ecologically relevant genetic variation from a non-*Arabidopsis* perspective. *Current Opinion in Plant Biology* **11**: 156–162.

Kawagoe T, Kudoh H. 2010. Escape from floral herbivory by early flowering in *Arabidopsis halleri* subsp. *gemmifera*. *Oecologia* **164**: 713–720.

Kitano H. 2002. Systems biology: a brief overview. *Science* **295**: 1662–1664.

Kitano J, Bolnick DI, Beauchamp DA, *et al*. 2008. Reverse evolution of armor plates in the threespine stickleback. *Current Biology* **18**: 769–774.

Kobayashi MJ, Shimizu KK. 2011. Challenges in the studies on flowering time: interfaces between phenological researches and the molecular network of flowering genes. *Ecological Research*, doi:10.1007/s11284-011-0835-2.

Koch MA, Haubold B, Mitchell-Olds T. 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis, Arabis*, and related genera (Brassicaceae). *Molecular Biology and Evolution* **17**: 1483–1498.

Korner C, Basler D. 2010. Phenology under global warming. *Science* **327**: 1461–1462.

Kumar SV, Wigge PA. 2010. H2A.Z-containing nucleosomes mediate the thermosensory response in arabidopsis. *Cell* **140**: 136–147.

Kusaba M, Dwyer K, Hendershot J, Vrebalov J, Nasrallah JB, Nasrallah ME. 2001. Self-incompatibility in the genus *Arabidopsis*: characterization of the *S* locus in the outcrossing *A. lyrata* and its autogamous relative *A. thaliana*. *The Plant Cell* **13**: 627–643.

Lande R, Schemske DW. 1985. The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. *Evolution* **39**: 24–40.

Leakey ADB, Ainsworth EA, Bernard SM, *et al*. 2009. Gene expression profiling: opening the black box of plant ecosystem responses to global change. *Global Change Biology* **15**: 1201–1213.

Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D. 2005. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PloS Genetics* **1**: e6. doi:10.1371/journal.pgen.0010006.

Liu P, Sherman-Broyles S, Nasrallah ME, Nasrallah JB. 2007. A cryptic modifier causing transient self-incompatibility in *Arabidopsis thaliana*. *Current Biology* **17**: 734–740.

Llaurens V, Billiard S, Leducq JB, Castric V, Klein EK, Vekemans X. 2008. Does frequency-dependent selection with complex dominance interactions accurately predict allelic frequencies at the self-incompatibility locus in *Arabidopsis halleri*? *Evolution* **62**: 2545–2557.

Mable BK. 2008. Genetic causes and consequences of the breakdown of self-incompatibility: case studies in the Brassicaceae. *Genetics Research* **90**: 47–60.

Mable BK, Schierup MH, Charlesworth D. 2003. Estimating the number, frequency, and dominance of *S*-alleles in a natural population of *Arabidopsis lyrata* (Brassicaceae) with sporophytic control of self-incompatibility. *Heredity* **90**: 422–431.

Meyer CL, Vitalis R, Saumitou-Laprade P, Castric V. 2009. Genomic pattern of adaptive divergence in *Arabidopsis halleri*, a model species for tolerance to heavy metal. *Molecular Ecology* **18**: 2050–2062.

Michaels SD, Amasino RM. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *The Plant Cell* **11**: 949–956.

Mitchell-Olds T. 2001. *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. *Trends in Ecology and Evolution* **16**: 693–700.

Mitchell-Olds T, Schmitt J. 2006. Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature* **441**: 947–952.

Nasrallah ME, Liu P, Nasrallah JB. 2002. Generation of self-incompatible *Arabidopsis thaliana* by transfer of two *S* locus genes from *A. lyrata*. *Science* **297**: 247–249.

de Nettancourt D. 2001. *Incompatibility and incongruity in wild and cultivated plants*. Berlin: Springer.

Newbigin E, Uyenoyama MK. 2005. The evolutionary dynamics of self-incompatibility systems. *Trends in Genetics* **21**: 500–505.

Nordborg M, Hu TT, Ishino Y *et al*. 2005. The pattern of polymorphism in *Arabidopsis thaliana*. *PLoS Biology* **3**: e196. doi:10.1371/journal.pbio.0030196.

Okamoto S, Odashima M, Fujimoto R, Sato Y, Kitashiba H, Nishio T. 2007. Self-compatibility in *Brassica napus* is caused by independent mutations in *S*-locus genes. *The Plant Journal* **50**: 391–400.

Ossowski S, Schneeberger K, Lucas-Lledo JI, *et al*. 2010. The rate and molecular spectrum of spontaneous mutations in *Arabidopsis thaliana*. *Science* **327**: 92–94.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* **37**: 637–669.

Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42.

Platt A, Horton M, Huang YS, *et al*. 2010. The scale of population structure in *Arabidopsis thaliana*. *PLoS Genetics* **6**: e1000843. doi:10.1371/journal.pgen.1000843.

Prigoda NL, Nassuth A, Mable BK. 2005. Phenotypic and genotypic expression of self-incompatibility haplotypes in *Arabidopsis lyrata* suggests unique origin of alleles in different dominance classes. *Molecular Biology and Evolution* **22**: 1609–1620.

Quintana-Murci L, Alcais A, Abel L, Casanova JL. 2007. Immunology *in natura*: clinical, epidemiological and evolutionary genetics of infectious diseases. *Nature Immunology* **8**: 1165–1171.

Richards CL, Hanzawa Y, Katari MS, Ehrenreich IM, Engelmann KE, Purugganan MD. 2009. Perspectives on ecological and evolutionary systems biology. *Annual Plant Reviews* **35**: 331–349.

Roosens NH, Willems G, Saumitou-Laprade P. 2008. Using *Arabidopsis* to explore zinc tolerance and hyperaccumulation. *Trends in Plant Science* **13**: 208–215.

Satake A. 2004. Modeling spatial dynamics of episodic and synchronous reproduction by plant populations: the effect of small-scale pollen coupling and large-scale climate. *Population Ecology* **46**: 119–128.

Schmickl R, Jorgensen MH, Brysting AK, Koch MA. 2010. The evolutionary history of the *Arabidopsis lyrata* complex: a hybrid in the amphi-Beringian area closes a large distribution gap and builds up a genetic barrier. *BMC Evolutionary Biology* **10**: 98. doi:10.1186/1471-2148-10-98.

Schmid K, Torjek O, Meyer R, Schmuths H, Hoffmann MH, Altmann T. 2006. Evidence for a large-scale population structure of *Arabidopsis thaliana* from genome-wide single nucleotide polymorphism markers. *Theoretical and Applied Genetics* **112**: 1104–1114.

Schoener TW. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* **331**: 426–429.

Schopfer CR, Nasrallah ME, Nasrallah JB. 1999. The male determinant of self-incompatibility in *Brassica*. *Science* **286**: 1697–1700.

Sharbel TF, Haubold B, Mitchell-Olds T. 2000. Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Molecular Ecology* **9**: 2109–2118.

Sheldon CC, Burn JE, Perez PP, *et al*. 1999. The *FLF MADS* box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *The Plant Cell* **11**: 445–458.

Sherman-Broyles S, Boggs N, Farkas A, *et al*. 2007. *S* locus genes and the evolution of self-fertility in *Arabidopsis thaliana*. *The Plant Cell* **19**: 94–106.

Shiba H, Kakizaki T, Iwano M, Tarutani Y, Watanabe M, Isogai A, Takayama S. 2006. Dominance relationships between self-incompatibility alleles controlled by DNA methylation. *Nature Genetics* **38**: 297–299.

Shimizu KK. 2002. Ecology meets molecular genetics in *Arabidopsis*. *Population Ecology* **44**: 221–233.

Shimizu KK, Purugganan MD. 2005. Evolutionary and ecological genomics of Arabidopsis. *Plant Physiology* **138**: 578–584.

Shimizu KK, Fujii S, Marhold K, Watanabe K, Kudoh H. 2005. *Arabidopsis kamchatica* (Fisch. ex DC.) K. Shimizu & Kudoh and *A. kamchatica* subsp. *kawasakiana* (Makino) K. Shimizu & Kudoh, new combinations. *Acta Phytotaxonomica et Geobotanica* **56**: 165–174.

Shimizu KK, Shimizu-Inatsugi R, Tsuchimatsu T, Purugganan MD. 2008. Independent origins of self-compatibility in *Arabidopsis thaliana*. *Molecular Ecology* **17**: 704–714.

Shimizu-Inatsugi R, Lihova J, Iwanaga H, *et al*. 2009. The allopolyploid *Arabidopsis kamchatica* originated from multiple individuals of *Arabidopsis lyrata* and *Arabidopsis halleri*. *Molecular Ecology* **18**: 4024–4048.

Stebbins GL. 1974.*Flowering plants: evolution above the species level*. Cambridge, MA: Harvard University Press.

Stinchcombe JR, Weinig C, Ungerer M, *et al*. 2004. A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene *FRIGIDA*. *Proceedings of the National Academy of Sciences of the USA* **101**: 4712–4717.

Sugisaka J, Kudoh H. 2008. Breeding system of the annual Cruciferae, *Arabidopsis kamchatica* subsp. *kawasakiana*. *Journal of Plant Research* **121**: 65–68.

Sung S, Amasino RM. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* **427**: 159–164.

Suzuki G, Kai N, Hirose T, *et al*. 1999. Genomic organization of the *S* locus: identification and characterization of genes in *SLG/SRK* region of S^9 haplotype of *Brassica campestris* (syn. *rapa*). *Genetics* **153**: 391–400.

Takasaki T, Hatakeyama K, Suzuki G, Watanabe M, Isogai A, Hinata K. 2000. The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. *Nature* **403**: 913–916.

Takayama S, Isogai A. 2005. Self-incompatibility in plants. *Annual Review of Plant Biology* **56**: 467–489.

Takayama S, Shiba H, Iwano M, *et al*. 2000. The pollen determinant of self-incompatibility in *Brassica campestris*. *Proceedings of the National Academy of Sciences of the USA* **97**: 1920–1925.

Takayama S, Shimosato H, Shiba H, *et al*. 2001. Direct ligand–receptor complex interaction controls *Brassica* self-incompatibility. *Nature* **413**: 534–538.

Takebayashi N, Morrell PL. 2001. Is self-fertilization an evolutionary dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *American Journal of Botany* **88**: 1143–1150.

Tang CL, Toomajian C, Sherman-Broyles S, *et al*. 2007. The evolution of selfing in *Arabidopsis thaliana*. *Science* **317**: 1070–1072.

Tantikanjana T, Rizvi N, Nasrallah ME, Nasrallah JB. 2009. A dual role for the *S*-locus receptor kinase in self-incompatibility and pistil development revealed by an *Arabidopsis rdr6* mutation. *The Plant Cell* **21**: 2642–2654.

Tarutani Y, Shiba H, Iwano M, *et al*. 2010. *Trans*-acting small RNA determines dominance relationships in *Brassica* self-incompatibility. *Nature* **466**: 983–986.

Toth AL, Varala K, Newman TC, *et al*. 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science* **318**: 441–444.

Tsuchimatsu T, Suwabe K, Shimizu-Inatsugi R, *et al*. 2010. Evolution of self-compatibility in *Arabidopsis* by a mutation in the male specificity gene. *Nature* **464**: 1342–1346.

Uyenoyama MK, Zhang Y, Newbigin E. 2001. On the origin of self-incompatibility haplotypes: transition through self-compatible intermediates. *Genetics* **157**: 1805–1817.

Van Rossum F, Bonnin I, Fenart S, Pauwels M, Petit D, Saumitou-Laprade P. 2004. Spatial genetic structure within a metallicolous population of *Arabidopsis halleri*, a clonal, self-incompatible and heavy-metal-tolerant species. *Molecular Ecology* **13**: 2959–2967.

Wang R, Farrona S, Vincent C, *et al*. 2009. *PEP1* regulates perennial flowering in *Arabis alpina*. *Nature* **459**: 423–427.

Weinig C, Ungerer MC, Dorn LA, *et al*. 2002. Novel loci control variation in reproductive timing in *Arabidopsis thaliana* in natural environments. *Genetics* **162**: 1875–1884.

Westerman JM, Lawrence MJ. 1970. Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana*. I. Inbred lines: description. *Heredity* **25**: 609–627.

Whitham TG, Bailey JK, Schweitzer JA, *et al*. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* **7**: 510–523.

Wilczek AM, Roe JL, Knapp MC, *et al*. 2009. Effects of genetic perturbation on seasonal life history plasticity. *Science* **323**: 930–934.

Wilczek AM, Burghardt LT, Cobb AR, Cooper MD, Welch SM, Schmitt J. 2010. Genetic and physiological bases for phenological responses to current and predicted climates. *Philosophical Transactions of the Royal Society Series B – Biological Sciences* **365**: 3129–3147.

Zufall RA, Rausher MD. 2004. Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature* **428**: 847–850.