

Chapter 1

New Insights on Heterostyly: Comparative Biology, Ecology and Genetics

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Abstract Here, we review recent progress on the evolutionary history, functional ecology, genetics and molecular biology of heterostyly using a variety of taxa to illustrate advances in understanding. Distyly and tristily represent remarkable examples of convergent evolution and are represented in at least 28 flowering plant families. The floral polymorphisms promote disassortative mating and are maintained in populations by negative frequency-dependent selection. Comparative analyses using phylogenies and character reconstruction demonstrate multiple independent origins of heterostyly and the pathways of evolution in several groups. Field studies of pollen transport support the Darwinian hypothesis that the reciprocal style–stamen polymorphism functions to increase the proficiency of animal-mediated cross-pollination. Although the patterns of inheritance of the style morphs are well established in diverse taxa, the identity, number and organization of genes controlling the heterostylous syndrome are unknown, despite recent progress. In future, it will be particularly important to establish the contribution of ‘supergenes’ vs. regulatory loci that cause morph-limited expression of genes.

Abbreviations

2D PAGE	Two-dimensional polyacrylamide gel electrophoresis
<i>GPA</i> locus	Gynoecium, Pollen size, Anther height
IEF	Isoelectric focussing
L-morph	Long-styled morph

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M-morph	Mid-styled morph
mRNA	messenger RNA
<i>P*</i>	Abnormal pin morph
<i>PvSLL1</i>	cDNA from <i>Primula</i> linked to the <i>S-locus</i> ; encodes a small putative transmembrane protein of unknown function
<i>PvSLL2</i>	cDNA from <i>Primula</i> closely linked to the <i>S-locus</i> ; has homology to the <i>CONSTANS-LIKE</i> gene
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SI	Self-incompatibility
<i>S-locus</i>	Self-incompatibility locus
S-morph	Short-styled morph

1.1 Introduction

The sexual organs of most flowering plants exhibit a small degree of continuous variation resulting from quantitative inheritance and environmental influences. A strikingly different pattern of variation is evident in populations of some species, where hermaphroditic individuals fall into two or three morphologically distinct mating groups, which differ in style length, anther height and a suite of ancillary pollen and stigma polymorphisms (Darwin 1877; Vuilleumier 1967; Ganders 1979; Barrett 1992). Populations with this type of polymorphic sexual variation are distylous or tristylous, respectively, and the general condition is referred to as heterostyly.

The defining feature of heterostylous populations is a reciprocal arrangement of sex-organ heights in the floral morphs (Fig. 1.1), also known as reciprocal herkogamy (Webb and Lloyd 1986). By convention, the morphs are referred to as long- and short-styled (hereafter L- and S-morphs) in distylous populations, and long-, mid- and short-styled (hereafter L-, M- and S-morphs) in tristylous populations. In most heterostylous species, reciprocal herkogamy is associated with a heteromorphic self-incompatibility (SI) system that limits or prevents self- and intra-morph mating. Therefore, compatible pollinations occur only between anthers and stigmas of equivalent height, termed 'legitimate pollinations' (Fig. 1.1; Darwin 1877). Understanding the evolution, function and genetic basis of heterostyly has attracted considerable attention since Darwin's classic book on polymorphic sexual systems in plants (Darwin 1877). The sustained fascination with heterostyly occurs because the sexual polymorphisms are a remarkable example of convergent evolution. In addition, they represent one of the classic research paradigms for the study of evolution and adaptation in plants (Barrett 1992).

Darwin (1877) provided the earliest adaptive explanation for the function of heterostyly. He proposed that the reciprocal placement of anthers and stigmas was a mechanism to promote pollinator-mediated cross-pollination between floral morphs.

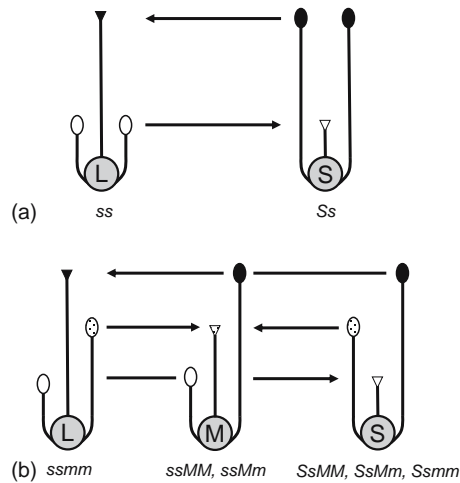


Fig. 1.1 The heterostylous floral polymorphisms: (a) distyly and (b) tristyly. L, M and S refer to the long-, mid- and short-styled morphs, respectively. The arrows indicate cross-pollinations between anthers and stigmas of equivalent height. In the majority of heterostylous species these are the only compatible pollinations. Genotypes for the floral morphs with the most common patterns of inheritance are indicated (see Sect. 1.4.1. for further details)

According to Darwin's hypothesis, pollinators visiting heterostylous flowers pick up pollen on different parts of their bodies during nectar feeding, and cross-pollen transfer between floral morphs is promoted by this segregated pollen deposition. Several lines of evidence support Darwin's cross-pollination hypothesis (Kohn and Barrett 1992; Lloyd and Webb 1992b) and heterostyly is generally described as an 'outcrossing mechanism'. However, this interpretation is insufficient for two reasons. First, self-incompatibility (SI) in heterostylous populations guarantees outcrossing, and, second, by preventing intra-morph mating heteromorphic SI restricts mating opportunities with one half (distyly), or one third (tristyly), of the plants in a population. A more complete interpretation of the adaptive significance of heterostyly recognizes different functional roles for the morphological and physiological components of the polymorphism in promoting fitness through male and female function, respectively.

Reciprocal herkogamy, as Darwin (1877) proposed, functions to promote proficient cross-pollination by reducing male gamete wastage on incompatible stigmas and increasing fitness through male function (Lloyd and Webb, 1992a, b). In contrast, SI safeguards against self-fertilization and inbreeding depression, thereby promoting the maternal component of fitness. Recognition of these different paternal and maternal functions resolves the apparent redundancy of two mechanisms with essentially the same role. The evolution of heterostyly reduces the conflict that can occur in sexually monomorphic animal-pollinated species—achieving efficient cross-pollination but simultaneously avoiding self-interference between female and male sexual organs (Barrett 2002).

Studies of heterostyly have largely concentrated on a few well-characterized taxa (e.g. *Primula*, *Linum*, *Lythrum*) originally studied by Darwin (1877). Of these, *Primula* has attracted most attention and is often represented in the literature as the model system for heterostyly (Mast and Conti 2006; see also Chap. 14, this volume). However, heterostyly is now reported from at least 28 angiosperm families and the polymorphism has evolved on numerous occasions. In addition, other stylar polymorphisms have also been recognized with their own distinctive features [e.g. stigma-height dimorphism (Baker et al. 2000a, b; Barrett et al. 2000a); enantiostyly (Barrett 2002; Jesson and Barrett 2003); flexistyly (Li et al. 2001; Renner 2001; Sun et al. 2007); inversostyly (Pauw 2005)] raising new questions about their evolution, function and relation to heterostyly.

Early research on heterostyly largely focused on genetical aspects of the polymorphism. Indeed, fundamental concepts in Mendelian and population genetics, including patterns of inheritance, linkage, supergenes, epistasis and polymorphic equilibria, were initially studied in *Primula* and *Lythrum* by leading geneticists, including W. Bateson, R.A. Fisher, J.B.S. Haldane, A. Ernst, A.B. Stout, K. Mather and D. Lewis. Today, a much broader range of questions are being addressed in heterostylous species employing diverse approaches. The objective of this chapter is to review recent advances on the study of heterostyly by examining progress made since the last general treatment (Barrett 1992). We review work on the comparative biology, ecology and genetics of heterostyly and conclude by briefly outlining future research for solving outstanding problems remaining in the study of heterostylous plants. A goal of our review is to demonstrate that although heterostyly is probably the most well studied plant sexual polymorphism, there still remain many unanswered questions that require future investigation.

1.2 Comparative Biology and Evolutionary History of Heterostyly

Heterostyly has a scattered distribution among at least 28 angiosperm families with new heterostylous taxa continually reported [e.g. distyly in *Aliciella* formerly *Gillia* (Polemoniaceae; Tommerup 2001); *Salvia* (Lamiaceae; Barrett et al. 2000b); and *Tylosema* (Caesalpinioideae; Hartley et al. 2002); tristyly in *Hugonia* (Linaceae; Thompson et al. 1996)]. Lloyd and Webb (1992a) surveyed the character states of 25 families with heterostylous species to identify why the polymorphism may have evolved in some families and not others. Their analysis indicated that there are constraints on the types of flowers in which reciprocal herkogamy is likely to evolve. Heterostylous flowers are usually actinomorphic with a simple open corolla and a floral tube with nectar concealed at the base. These flowers are described as stereomorphic or 'depth-probed' (Lloyd and Webb 1992a), with sexual organs contacted by long-tongued pollinators in succession during nectar feeding. Heterostyly is rarely associated with strongly zygomorphic flowers, probably because in such groups effective cross-pollen transfer is achieved through pollinator positioning.

Families in which flowers possess numerous stamens, free carpels, open-dished shaped corollas and exposed nectar usually lack the precision in pollen transfer required for the evolution of reciprocal herkogamy. However, exceptions to these patterns exist in heterostylous taxa [e.g. zygomorphy in *Salvia* (Barrett et al. 2000b); open dish- or bowl-shaped flowers in *Fagopyrum* (Bjorkman 1995) and *Turnera* (Rama Swamy and Bahadur 1984); numerous stamens and nectar-less flowers in *Hypericum* (Ornduff 1975)], raising the question of how reciprocal herkogamy evolved and is maintained in these taxa.

1.2.1 Phylogeny Reconstruction and Character Evolution

The advent of molecular systematics, phylogeny reconstruction and character mapping has led to interest in the evolutionary history of SI. See Chap. 4 for consideration of the evolutionary history of homomorphic SI. Here, we consider studies of heterostylous taxa, which include Pontederiaceae (Kohn et al. 1996), *Amsinckia* (Schoen et al. 1997), *Houstonia* (Church 2003); *Primula* (Mast et al. 2004), *Narcissus* (Graham and Barrett 2004); *Linum* (Armbruster et al. 2006), *Turnera* (Truyens et al. 2005) and Lythraceae (Morris 2007). Phylogenetic analyses of these groups have been conducted to address questions concerning the origin and evolutionary history of heterostyly and related sexual systems, and these have included the following: (1) Has heterostyly evolved more than once in a particular lineage? (2) What are the ancestral states and intermediate stages involved in the evolution of heterostyly? (3) What is the order of establishment of morphological and physiological traits in the heterostyous syndrome? (4) What are the evolutionary relationships between heterostyly and related stilar conditions, including homostyly? Part of this work has been motivated by efforts to distinguish between the predictions of competing theoretical models on the evolution of heterostyly (Charlesworth and Charlesworth 1979a; Lloyd and Webb 1992a, b).

Lloyd and Webb (1992a) estimated that heterostyly originated on at least 23 separate occasions, based on its distribution among 19 orders of flowering plants, but conceded that many more origins may be involved if multiple origins have occurred within heterostylous taxa. Although several studies have assumed that heterostyly is the basal condition in lineages (Schoen et al. 1997; Tuyens et al. 2005), other evidence points to multiple origins of heterostyly within some genera (Graham and Barrett 2004). Inferences on the number of origins of heterostyly within particular groups may be particularly sensitive to taxon sampling and the weighting schemes employed for the gain and loss of heterostyly [e.g. Pontederiaceae (Kohn et al. 1996); *Linum* (Armbruster et al. 2006); *Primula* (Mast et al. 2006)]. In large geographically widespread families with numerous heterostylous species, such as Rubiaceae and Oxalidaceae, the polymorphism may have had multiple origins. If this turns out to be the case, it will be interesting to investigate the details of each transition, including their ecological and developmental basis and if the patterns of inheritance are similar.