

## THE GENETIC STRUCTURE OF ORCHID POPULATIONS AND ITS EVOLUTIONARY IMPORTANCE

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Evolution through either natural selection or genetic drift is dependent on variation at the genetic and morphological levels. Processes that influence the genetic structure of populations include mating systems, effective population size, mutation rates and gene flow among populations. We investigated the patterns of population genetic structure of orchids and evaluated if evolutionary processes are more likely at the individual population level than at the multipopulation/species level. We hypothesized that because orchid populations are frequently small and reproductive success is often skewed, we should observe many orchids with high population genetic substructure suggesting limited gene flow among populations. If limited gene flow among populations is a common pattern in orchids, then it may well be an important component that affects the likelihood of genetic drift and selection at the local population level. Such changes may lead to differentiation and evolutionary diversification.

A main component in evolutionary processes is the necessary condition of isolation. The amount of gene flow among local populations will determine whether or not individual populations (demes) can evolve independently which may lead to cladogenesis. Usually one migrant per generation is sufficient to prevent populations from evolving independently from other populations when effective population sizes are large. Theoretically, if the gene flow rate,  $Nm$  (the effective number of migrants per generation;  $N$  = effective population size,  $m$  = migration rate), is larger than two individuals per generation, then it is sufficient to prevent local adaptation while gene flow less than one per generation will likely result in population differentiation by selection or genetic drift (Merrell 1981, Roughgarden 1996). If  $Nm$  lies between one and two, there will be considerable variation in gene frequencies among populations (Merrell 1981). Consequently,

populations will have similar genetic structure as if mating were panmictic ( $Nm > 2$ ). Alternatively, if gene flow is low ( $Nm < 1$ ), populations will have different genetic structures that may result in evolutionary change through either adaptation to the local environments via natural selection or through random effects such as genetic drift.

Direct observation of gene flow can be viewed by the use of mark and recapture studies (for mobile organisms, or stained pollen) or tracking marker alleles (paternity analysis) over a short number of generations. Few orchid studies have attempted to directly observe gene flow and thus far only staining or micro-tagging pollinaria have been used (Peakall 1989, Nilsson *et al.* 1992, Folsom 1994, Tremblay 1994, Salguero-Faría & Ackerman 1999). All these studies examined gene flow only within populations.

Indirect methods for detecting gene flow are obtained from allele frequencies and are an estimate of the average long-term effect of genetic differentiation by genetic drift. The alleles are assumed to be neutral so that genetic differentiation based on these markers would be a consequence of drift rather than natural selection. Bohomak (1999) concluded that simple population genetic statistics are robust for inferring gene flow among groups of individuals.

The most common approach is the degree of population differentiation at the genetic level using Wright's  $F$  estimates on data obtained through protein electrophoresis or various PCR type approaches. The  $F$  statistics separate the amount of genetic variation which can be attributed to inbreeding among closely related individuals in a population:  $F_{IS}$  is the inbreeding coefficient within individuals;  $F_{IT}$  is the result of non random mating within a population and the effect of population subdivision; and a third statistic,  $F_{ST}$ , is the fixation index due to random genetic drift and the lack of panmixia among populations (Wright 1978).

Table 1. Estimates of gene flow in orchids. Nm(W) = gene flow estimates based on Wright's statistics; Gst coefficient of genic differentiation among populations. <sup>1</sup> Nm calculated by the present authors from Gst or Fst using formula on p. 320 of Hartl & Clark (1989). <sup>2</sup> Recalculated using previous formula, original Nm value 3.70. <sup>3</sup> Calculated from RAPD markers. <sup>4</sup> Calculated from cpDNA. <sup>5</sup> No genetic differentiation found among populations. <sup>6</sup> Calculated according to Weir and Cockerham's statistics. <sup>7</sup> Estimated using RAPD's and AMOVA.

Species	References	Nm(W)	Gst
<i>Calypso bulbosa</i> (L.) Oakes	Alexandersson & Ågren 2000	3.20	0.072
<i>Caladenia tentaculata</i> Tate	Peakall & Beattie 1996	7.10 <sup>1</sup>	0.0346
<i>Cephalanthera damasonium</i> (Mill.) Druce	Scacchi, De Angelis & Corbo 1991	--5	--5
<i>Cephalanthera longifolia</i> (L.) Fritsch	Scacchi, De Angelis & Corbo 1991	2.15 <sup>1</sup>	0.104
<i>Cephalanthera rubra</i> (L.) Rich.	Scacchi, De Angelis & Corbo 1991	0.76 <sup>1</sup>	0.247
<i>Cymbidium goeringii</i> Rchb. f.	Chung & Chung 1999	2.30	0.098
<i>Cypripedium acaule</i> Ait.	Case 1994	1.27 <sup>1</sup>	0.164
<i>Cypripedium calceolus</i> L.	Case 1993, 1994	1.63 <sup>1</sup>	0.196
<i>Cypripedium candidum</i> Muhl. ex Willd.	Case 1994	3.37 <sup>1</sup>	0.069
<i>Cypripedium fasciculatum</i> Kellogg ex S. Watson	Aagaard, Harrod & Shea 1999	6.00	0.04
<i>Cypripedium kentuckiense</i> C. F. Reed	Case <i>et al.</i> 1998	1.12 <sup>1</sup>	0.182
<i>Cypripedium parviflorum</i> Salisb.			
var. <i>pubescens</i> (Willd.) O. W. Knight	Case <i>et al.</i> 1998	1.28 <sup>1</sup>	0.163
Southern populations	Wallace & Case 2000	0.94	0.209
Northern populations		1.57	0.137
var. <i>makasin</i> (Farw.) Sheviak		1.00	0.199
var. <i>parviflorum</i>		1.43	0.149
species level		0.83	0.232
<i>Cypripedium reginae</i> Walter	Case 1994	0.47 <sup>1</sup>	0.349
<i>Dactylorhiza romana</i> (Sebastiani) Soó	Bullini <i>et al.</i> 2001	3.32 <sup>1</sup>	0.07
<i>Dactylorhiza sambucina</i> (L.) Soó	Bullini <i>et al.</i> 2001	1.31 <sup>1</sup>	0.16
<i>Epidendrum conopseum</i> R. Br.	Bush, Kutz & Anderton 1999	1.43 <sup>3</sup>	0.149
<i>Epipactis helleborine</i> (L.) Crantz	Scacchi, Lanzara & De Angelis 1987	7.3 <sup>1</sup>	0.033
European populations	Squirrell <i>et al.</i> , 2001	1.00 <sup>1</sup>	0.200
North American	Hollingsworth & Dickson 1997	0.24 <sup>1,4</sup>	0.506 <sup>4</sup>
		2.53 <sup>1</sup>	0.240
		0.791	
<i>Epipactis youngiana</i> Richards & Porter	Harris & Abbott 1997	2.431	0.093
<i>Eulophia sinensis</i> Miq.	Sun & Wong 2001	--	0.0
		0.133 <sup>1,3</sup>	0.653 <sup>3</sup>
<i>Gooyera procera</i> Ker-Gawl.	Wong & Sun 1999	0.221 <sup>1</sup>	0.523
		0.397 <sup>1,3</sup>	0.386 <sup>3</sup>
<i>Gymnadenia conopsea</i> (L.) R. Br.	Scacchi & De Angelis 1990	0.2801	0.471
<i>Gymnadenia conopsea</i> (L.) R. Br. <i>conopsea</i>	Soliva & Widmer 1999	2.96	0.078
<i>Gymnadenia conopsea</i> (L.) R. Br.			
subsp. <i>densiflora</i> (Wahl) E.G. Camus & A. Camus	Soliva & Widmer 1999	0.39	0.391
<i>Lepanthes caritensis</i> Tremblay & Ackerman	Carronero, Tremblay & Ackerman (unpublished)	1.30	0.167
<i>Lepanthes rupestris</i> Stimson	Tremblay & Ackerman 2001	1.84	0.170
<i>Lepanthes rubripetala</i> Stimson	Tremblay & Ackerman 2001	0.62	0.270
<i>Lepanthes eltoroensis</i> Stimson	Tremblay & Ackerman 2001	0.89	0.220
<i>Lepanthes sanguinea</i> Hook.	Carronero, Tremblay & Ackerman (unpublished)	1.45	0.144

Species	References	Nm(W)	Gst
<i>Lepanthes woodburyana</i> Stimson	Carronero, Tremblay & Ackerman (unpublished)	7.5	0.032
<i>Nigritella rhellicani</i> Teppner & Klein	Hedrén, Klein & Teppner 2000	1.381	0.153
<i>Orchis laxiflora</i> Lam.	Scacchi, De Angelis & Lanzara 1990	2.85 <sup>1</sup>	0.08
	Arduino <i>et al.</i> 1996	1.97 <sup>1</sup>	0.116
<i>Orchis longicornu</i> Poir.	Corrias <i>et al.</i> 1991	12.25 <sup>2</sup>	0.02
<i>Orchis mascula</i> (L.) L.	Scacchi, De Angelis & Lanzara 1990	2.761	0.083
<i>Orchis morio</i> L.	Scacchi, De Angelis & Lanzara 1990	3.66 <sup>1</sup>	0.064
	Rossi <i>et al.</i> 1992	4.75 <sup>1</sup>	0.05
<i>Orchis papilionacea</i> L.	Scacchi, De Angelis & Lanzara, 1990	6.33 <sup>1</sup>	0.038
<i>Orchis palustris</i> Jacq.	Arduino <i>et al.</i> 1996	0.31 <sup>1</sup>	0.448
<i>Orchis pauciflora</i> Ten.	Scacchi, De Angelis & Lanzara 1990	6.00 <sup>1</sup>	0.040
<i>Orchis provincialis</i> Balb.	Scacchi, De Angelis & Lanzara 1990	10.62 <sup>1</sup>	0.023
<i>Orchis purpurea</i> Huds.	Scacchi, De Angelis & Lanzara 1990	5.70 <sup>1</sup>	0.042
<i>Orchis tridentata</i> Scop.	Scacchi, De Angelis & Lanzara 1990	6.16 <sup>1</sup>	0.039
<i>Paphiopedilum micranthum</i> T. Tang & F. T. Wang	Li, Luo & Ge 2002	0.06 <sup>1</sup>	0.797 <sup>7</sup>
<i>Platanthera leucopaea</i> (Nutt.) Lindl.	Wallace 2002	0.08 <sup>1</sup>	0.754
		0.71 <sup>1</sup>	0.26 <sup>3</sup>
<i>Pterostylis</i> aff. <i>alata</i> (Labill.) Rchb.f.	Sharma <i>et al.</i> 2001	0.81 <sup>1</sup>	0.235
<i>Pterostylis angusta</i> A.S. George	Sharma <i>et al.</i> 2001	1.30 <sup>1</sup>	0.161
<i>Pterostylis aspera</i> D. L. Jones & M. A. Clem.	Sharma <i>et al.</i> 2001	1.01 <sup>1</sup>	0.198
<i>Pterostylis gibbosa</i> R. Br.	Sharma, Clements & Jones 2000	1.42	0.15
<i>Pterostylis hamiltonii</i> Nicholls	Sharma <i>et al.</i> 2001	0.86 <sup>1</sup>	0.225
<i>Pterostylis rogersii</i> E. Coleman	Sharma <i>et al.</i> 2001	1.10 <sup>1</sup>	0.186
<i>Pterostylis scabra</i> Lindl.	Sharma <i>et al.</i> 2001	0.83 <sup>1</sup>	0.232
<i>Spiranthes diluvialis</i> Sheviak	Arft & Ranker 1998	5.44	0.044
<i>Spiranthes sinensis</i> (Pers.) Ames	Sun 1996	1.19	0.174
<i>Spiranthes hongkongensis</i> S. H. Hu & Barretto	Sun 1996	-- <sup>5</sup>	-- <sup>5</sup>
<i>Tipularia discolor</i> (Pursh) Nutt.	Smith, Hunter & Hunter 2002	0.35 <sup>7</sup>	0.415
<i>Tolumnia variegata</i> (Sw.) Braem	Ackerman & Ward 1999	2.50	0.09
<i>Vanilla claviculata</i> (W. Wright) Sw.	Nielsen & Siegismund 1999	1.33	0.158
<i>Vanilla barbellata</i> Rchb. f.	Nielsen & Siegismund 1999	1.78	0.123
<i>Zeuxine gracilis</i> Blume	Sun & Wong 2001	0.500 <sup>1</sup>	0.333
		0.214 <sup>1</sup>	0.539 <sup>3</sup>
<i>Zeuxine strateumatica</i> Schltr.	Sun & Wong 2001	0.021 <sup>3</sup>	0.924 <sup>3</sup>

Consequently, if we make the assumption that the genetic markers sampled are neutral or nearly neutral and that the observed level of FST is a measure of the current gene flow among populations (rather than a historical remnant), then we can evaluate the likelihood that populations are effectively isolated. The scale of FST is from 0 (no population subdivision) to 1.0 (complete genetic differentiation among populations).

We gathered population genetic data for 58 species of terrestrial and epiphytic orchids from temperate and tropical species. The data are biased toward terrestrial/temperate species (N = 44). We found only

three studies of terrestrial/tropical species and ten epiphytic/tropical. There is also a bias toward certain taxa: *Orchis*, *Cypripedium*, *Pterostylis* and *Lepanthes* account for nearly half (30) of the 61 records (Table 1), 10 species of *Orchis*, 7 species each of *Cypripedium* and *Pterostylis*, 6 species of *Lepanthes*, 3 species of *Spiranthes*, *Epipactis*, *Cephalanthera* and *Gymnadenia*, 2 species of *Dactylorhiza*, *Epipactis*, *Vanilla* and *Zeuxine*, and one species each of *Caladenia*, *Calypso*, *Cymbidium*, *Epidendrum*, *Eulophia*, *Goodyera*, *Nigritella*, *Paphiopedilum*, *Platanthera*, *Tipularia*, and *Tolumnia*.

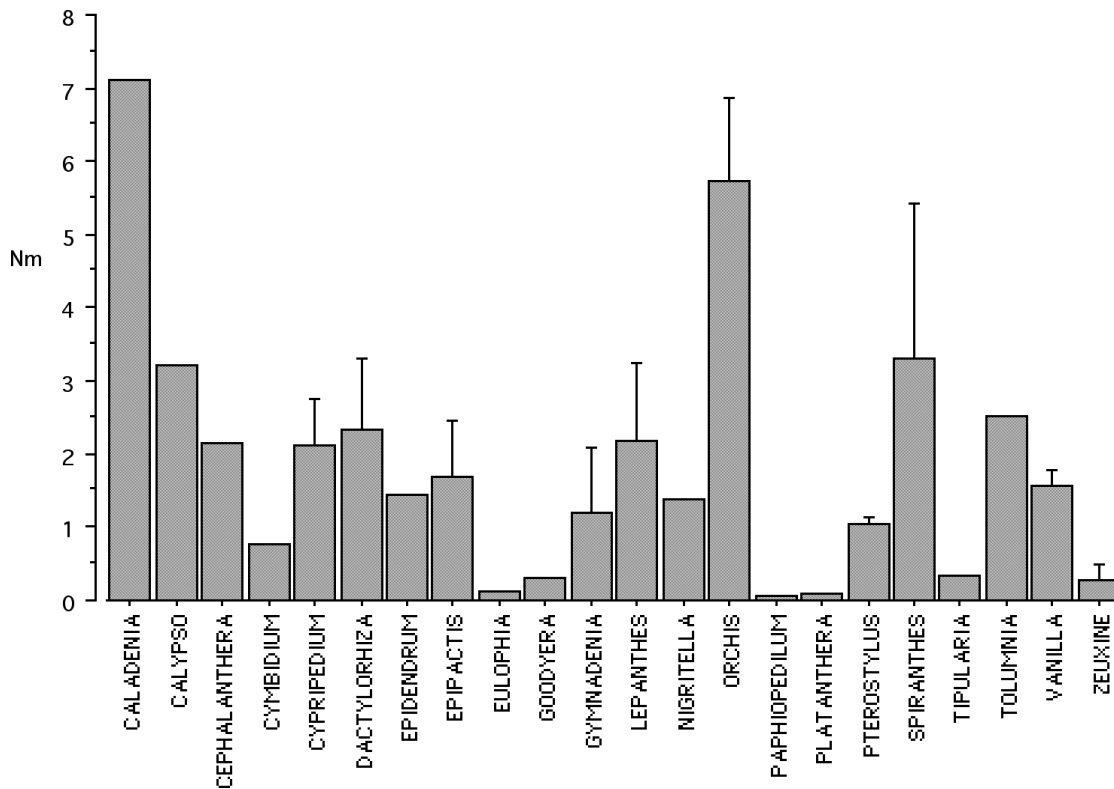


Figure 1: Distribution of mean (s.e.) gene flow (Nm) among genera of Orchids. Bars without error bars of single data points.

Gene flow among populations varies among species ranging from a high of 12 effective migrants per generation in *Orchis longicornu* (Corrias *et al.* 1991) to lows of less than 0.2 in *Zeuxine strateumatica* (Sun & Wong 2001). Assembling the species in groups based on their estimates of gene flow, we note that 18 species have less than one migrant per generation, while 19 species have more than two migrants per generation, and 17 of the species have a migration rates between one and two. No genetic differentiation was found among populations for *Cephalanthera damasonium* (Scacchi, De Angelis & Corbo 1991) and *Spiranthes hongkongensis* (Sun 1996). Consequently these two species are excluded from further analysis.

*Orchis* species typically have high estimates of gene flow among populations (Scacchi, De Angelis & Lanzara 1990, Corrias *et al.* 1991, Rossi *et al.* 1992) whereas *Lepanthes* and *Pterostylis* species have much lower gene flow estimates (Tremblay & Ackerman 2001, Sharma, Clements & Jones 2000; Sharma *et al.*

2001). However even within a genus variation in gene flow can be extensive (Table 1).

Are there phylogenetic associations with gene flow? The data for *Orchis* (mean Nm = 5.7), *Lepanthes* (mean Nm = 2.1) and *Pterostylis* (mean Nm = 1.0) are suggestive, but much more extensive sampling is needed for both temperate and tropical species. Curiously, *Lepanthes* and *Orchis* have very different population genetic parameters yet both are species-rich genera and are likely in a state of evolutionary flux. It seems to us that orchids have taken more than one expressway to diversification. For the group of species which has more than 2 migrants per generation local populations will not evolve independently, but as a group, consequently local morphological and genetic differences among groups will be wiped out, and populations will become homogeneous if gene flow continues at the level. When gene flow is high, selection studies from different populations should be evaluated together (Fig. 1).

For populations that have less than one migrant per

generation, local populations can evolve independently, and evolutionary studies should be done at the local level. In small populations, we may expect genetic drift to be present and selection coefficients should be high to counteract the effects of drift.

For species with intermediate gene flow it is probably wise to evaluate evolutionary processes at the local and multi-population/species level. We expect variance in migration rates to be large because of the skewed reproductive success among individuals, time periods and populations. Consequently, the outcome of the evolutionary process will likely depend on the amount and variation of the migration events and consistency in migration rates in time. If variance in gene flow through space and time is small, then the genetic differentiation will be more or less stable. But, for example, if variance in gene flow is high, with some periods having high gene flow followed by little or no gene flow for an extended period of time, it is possible that through natural selection and genetic drift local populations might differentiate sufficiently for cladogenesis during the period of reduced immigration.

Species with less than one migrant per population are basically unique evolutionary units evolving independently from other local populations. In populations with large  $N_e$  ( $> 50$ ), it is likely that natural selection will dominate evolutionary processes while if  $N_e$  is small ( $< 50$ ) genetic drift and selection can both be responsible for evolution. Consequently for these species, local adaptation to specific environmental conditions is possible.

This survey of population genetics studies of orchids shows that multiple evolutionary processes have likely been responsible for the remarkable diversification in orchids.

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