

# Hybridization and regional sex ratios in *Nemophila menziesii*

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## Abstract

I tested whether a region of high female frequencies in the gynodioecious plant, *Nemophila menziesii*, may be due to hybridization between regionally distributed populations with different corolla colours. I crossed plants in the greenhouse from populations with different corolla colours and found that hybrid crosses yielded higher frequencies of females than within-colour crosses. In the field, I found that populations with high female frequencies had intermediate mean corolla colours and higher variance in corolla colour, two traits suggesting hybridization. *Nemophila menziesii* has nuclear–cytoplasmic sex inheritance, thus if populations with different corolla colours are fixed for different male-sterile cytoplasms and matching nuclear restorer alleles, hybridization between populations with different corolla colour should yield high frequencies of females. Two populations that are all hermaphroditic in the field segregated females in hybrid crosses suggesting that field populations may contain sex ratio distorters but appear undistorted, a prediction of genomic conflict theory.

## Introduction

Genomic conflicts occur when one driving gene becomes overrepresented at the expense of other genes (Ostergen, 1945; Dawkins, 1976; Cosmides & Tooby, 1981; Hurst *et al.*, 1996). In plants, the most prominent and well-studied genomic conflict is the sex ratio distorter cytoplasmic male sterility (CMS). In hermaphroditic plants with CMS, maternally inherited mitochondrial genes that suppress the development of pollen tend to spread in populations because of a fitness advantage gained by either reallocation of resources from pollen to seeds or by inbreeding avoidance (Lewis, 1941). The spread of CMS genes and pollen abortion comes at the expense of biparentally inherited nuclear genes, thus selection is strong on nuclear genes to restore male fertility. Nuclear genes that suppress the action of the CMS genes and restore male fertility exist in all well-studied populations that contain CMS (reviewed in Frank, 1989).

Once nuclear restorer alleles enter a population, one theory predicts that they will go to fixation, barring any deleterious fitness effects (Charlesworth, 1981; Frank,

1989). Fixation of restorers returns the population to hermaphroditism until a new CMS gene enters the population either through mutation or migration, and the cycle repeats itself (Frank, 1989). Thus, populations that are entirely hermaphroditic but contain a diversity of hidden CMS and restorer genes may occur. This widespread presence of driving genes and fixed suppressors has been discussed theoretically (Frank, 1991; Hurst & Pomiankowski, 1991) but little empirical evidence exists (but see Atlan *et al.*, 1997).

Plant mitochondria experience frequent rearrangements which appear to lead to many different CMS genes (Belhassen *et al.*, 1993; Cuguen *et al.*, 1994; Samitoulaprade *et al.*, 1994; de Haan, 1997; Schnable & Wise, 1998; McCauley *et al.*, 2000). Because of this diversity, it is unlikely that isolated populations would become fixed for the same CMS types and their matching restorer alleles. If populations accumulate CMS cytotypes masked by their matching restorer alleles, then hybridization among populations should yield high female frequencies in the progeny because of a mismatch between CMS cytotypes and restorer alleles. Female frequency biases are common in hybrid crosses of agricultural species and varieties (Laser & Lersten, 1972; Kaul, 1988), however, much less work has been undergone investigating the effect of hybridization on the sex ratio in natural populations with CMS.

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In *Nemophila menziesii* H. & A., an annual plant with CMS, a unique regional pattern in both female frequencies and flower morphologies presents an opportunity to test whether hybridization in wild populations reinitiated a previous conflict. Female frequencies throughout most of the range of *N. menziesii* are low, <0.1. In the Southern North Coast Ranges (SNCR) of California, however, many populations have high female frequencies, up to 0.5. This region is also an area of overlap between plants with very blue corollas in Southern and Central California, and plants with very white corollas in Northern California and Oregon (Constance, 1941; Cruden, 1972; C. M. Barr, personal observation).

The overlap in corolla colour suggests that this region may be one in which hybridization between previously isolated populations of the different corolla colours has taken place. If populations of the different corolla colours have diverged for CMS genes and restorer alleles, then hybridization would result in mismatch between CMS types and their matching restorer alleles and thus could be the cause of the observed high female frequencies.

I tested this hypothesis using experimental greenhouse crosses and field correlations.

### Predictions from greenhouse crosses

If populations that have diverged for corolla colour have also diverged for male-sterile cytotypes and their matching restorer alleles, then greenhouse crosses between populations with white and blue corollas should yield higher frequencies of females than crosses between blue individuals or between white individuals.

In addition, if different corolla colours are associated with unique male-sterile cytoplasm, then reciprocal progeny sex ratios, which are direct tests for cytoplasmic differences (Grun, 1976), would differ between crosses between colour, but not within colour.

If the polymorphic populations are hybrids between white and blue populations, they would be more genetically related to either parent than the parents would be to each other. In greenhouse crosses, then, it might be expected that crosses between the polymorphic population and either of the presumed parents would experience fewer reproductive barriers and yield greater number of seeds than crosses between blue and white populations.

Finally, if populations have become fixed for male-sterile cytotypes, which are masked by fixed restorer alleles, crosses using plants from all hermaphroditic field populations as the maternal parent will yield female individuals.

### Predictions from field correlations

If hybridization has led to high female frequencies in the SNCR, then populations with high female frequencies should be expected to have two traits of hybrid popula-

tions: intermediate mean, and high variance in corolla colour as a result of backcrossing to parental types or later generation hybrid crosses.

## Methods

### Study system

*Nemophila menziesii* (Hydrophyllaceae) is an insect-pollinated, annual plant endemic to California and Oregon. Crosses show that male sterility is coded by at least two different cytoplasmic genes and that there exist at least two nuclear restorer genes (Ganders, 1978; C. M. Barr, unpublished data).

Flower colour is variable in *N. menziesii*, and ranges from all-white to all-blue, and colours are regionally distributed (Fig. 1). All flowers have a white centre that contains variable numbers of blue-purple dots.

I observed the regional pattern in sex ratio distribution by collecting sex ratio data from 68 populations throughout California between 1997 and 2002. Sex ratio data



**Fig. 1** Map of California showing the ranges of corolla colours and locations of the six populations from which seeds were collected and used in the greenhouse crossing experiment. Grey area = blue-flowered plants; white area = white-flowered plants; and hatched area = region of overlap with all-blue, all-white, and polymorphic populations. Blue and white corolla colour distributions taken from Cruden (1972). Circle colour indicates predominant corolla colour in population. Black = blue corollas; white = white corollas; hatched = polymorphic corollas.

were collected in two ways depending on the size of the population. In large populations (generally >500 individuals), I scored for sex all plants within a 1-m wide transect through the longest dimension of the population. For smaller populations, all flowering plants in the population were scored.

A subset of 22 populations was used for corolla colour measurements, and plants from six populations were used for greenhouse crosses.

### Predictions from greenhouse crosses

#### *Greenhouse crosses*

Two plants from each of six populations were grown in the greenhouses at the University of California, Irvine, and crossed in a diallel cross, with each plant crossed to every other plant. I chose two populations with blue corollas (Hastings and Hopland), three with white (CoveloTT, CoveloRM and Titlow Hill) and one with polymorphic corolla colours (North Beach). This sample size of plants is a small representation of the whole population, and was limited by the fact that the number of crosses in a diallel cross increases as a squared function of the number of plants, and can become prohibitively large. Figure 1 shows the location of each population. One individual from the CoveloTT population died partway through the experiment, thus there are results from only one plant from that population. All crosses are listed with maternal parent first, and paternal parent second.

A minimum of 10 flowers was crossed in each crossing combination. I emasculated flowers 3–5 days before pollination to prevent self-pollination, then applied fresh pollen to receptive stigmas using a toothpick. This method applies sufficient pollen to fertilize all available ovules (C. M. Barr, unpublished data).

#### *Within- and between-colour progeny sex ratios*

The frequency of females from greenhouse crosses between white × white plants, blue × blue, and blue × white were compared using a one-way Kruskal–Wallis test. Only crosses that had 10 or more progeny to sex were used in this analysis. All statistical analyses were performed using SAS (SAS Institute, 2001).

#### *Differences in male-sterile cytotypes*

I used reciprocal crosses and Monte Carlo simulations with 10 000 randomizations (Engels, 1988) to test whether individual plants within- and between-populations carry different male-sterile cytotypes. This is a direct test of cytoplasmic differences between pairs of plants to further clarify the general results from the within- and between-colour crosses. Because cytoplasmic genes are inherited mostly maternally (Mogensen, 1996), F<sub>1</sub> progeny of reciprocal crosses contain different cytoplasmic genes, but share on average 50% similar nuclear genomes. Therefore, differences in progeny sex ratios

are more likely due to cytoplasmic than to nuclear genes. Confirmation of cytoplasmic differences is generally achieved through backcrossing to the hybrid paternal genome, which gradually replaces the original paternal genome with that of the hybrid parent (Grun, 1976). Because *N. menziesii* is an annual plant, however, backcrosses are difficult and were not performed. I performed the Monte Carlo simulations on four pairs of between-colour crosses, one pair of blue × blue crosses, four pairs of blue × polymorphic crosses, seven pairs of white × polymorphic crosses, one white × white cross, and one polymorphic × polymorphic cross. The experiment-wise error rate was adjusted for multiple tests (separate crosses often shared one parent) using the formula  $1 - (1 - \alpha)^{1/k}$ , with  $k$  = number of tests (Sokal & Rohlf, 1995).

#### *Relative ability to produce hybrid seeds*

Two analyses were performed to determine whether greenhouse hybrid crosses with white or blue × polymorphic individuals (and reciprocal) produced more seeds per flower than crosses between blue × white (and reciprocal). Hybrid seed production may be used as one indication of genetic relatedness (in addition to other traits such as seed viability and plant growth), if geographical variants have differentiated for traits that affect hybrid seed number. Evidence of reduced in seed set in crosses between colours may suggest that populations of the two corolla types have diverged.

The first analysis was a two-way ANOVA using maternal and paternal corolla colour as main factors and their interaction. Two ANOVAs were then performed to test the relationships: (1) within-colour (blue × blue, white × white, polymorphic × polymorphic) > between-polymorphic [polymorphic × blue (and reciprocal), polymorphic × white (and reciprocal)] and (2) white/blue-polymorphic [polymorphic × blue (and reciprocal), polymorphic × white (and reciprocal)] > between-colour [blue × white (and reciprocal)].

#### *All-hermaphrodite populations as maternal parents in hybrid crosses*

The three white populations used in the above greenhouse crosses, CoveloTT, CoveloRM and Titlow Hill, have no female individuals in the field. Females segregating in crosses with plants from these populations used as maternal parents will indicate the presence of suppressed male-sterile cytotypes.

### Predictions from field correlations

#### *Quantifying corolla colour*

I ran a transect through the long dimension of each of 22 populations and collected one flower from the plant closest to each meter point. Thirty flowers were collected from each population. I recorded the sex of the plant, and then measured corolla colour by assigning



**Fig. 2** Sample flowers demonstrating colour categories based on petal pigment intensity and distribution. The colour categories refer only to the solid pigment on the petals, and not to the variable dots in the centre of the corolla (which is always white). 1, completely white or with a light purple hue corollas; 2, white corolla with blue venation; 3, white corolla with blue venation and blue scalloping on edges; 4, blue corolla with some white observable between blue veins; 5, completely blue corolla.

each flower into one of five categories based on (1) the intensity of the pigment, and (2) the patterning of the pigment. Completely white or white with light purple corollas were assigned a 1, and completely blue corollas were assigned a 5. Figure 2 shows example flowers for each of the five categories and category criteria.

#### *Corolla colour inheritance*

To determine whether hybrids between blue and white plants have corolla colours intermediate between the parents, I assigned colour categories to progeny from the diallel cross described above. Average corolla colour was compared between blue  $\times$  blue, white  $\times$  white and blue  $\times$  white/white  $\times$  blue crosses (crosses listed in Table 1) using a one-way Kruskal–Wallis test with cross type as the class variable, and average corolla colour as the dependent variable. Because crosses using plants from one blue population (Hastings) did not make any fertile seeds with any other population (see Results, Relative Ability to Produce Hybrid Seeds), progeny averages from only three within-blue crosses were used in this analysis. Averages from 11 hybrid crosses and seven within-white crosses were used.

#### *Colour average and variance regressions*

Regressions between population average corolla colour and population variance in corolla colour as independent variables and proportion females as the dependent variable were performed using 22 populations. Proportion females in each population were arcsin (square root) transformed to improve normality (Sokal & Rohlf, 1995). Quadratic regression analysis was performed with the corolla colour average data, and linear regression analysis was performed with the corolla colour variance data.

## Results

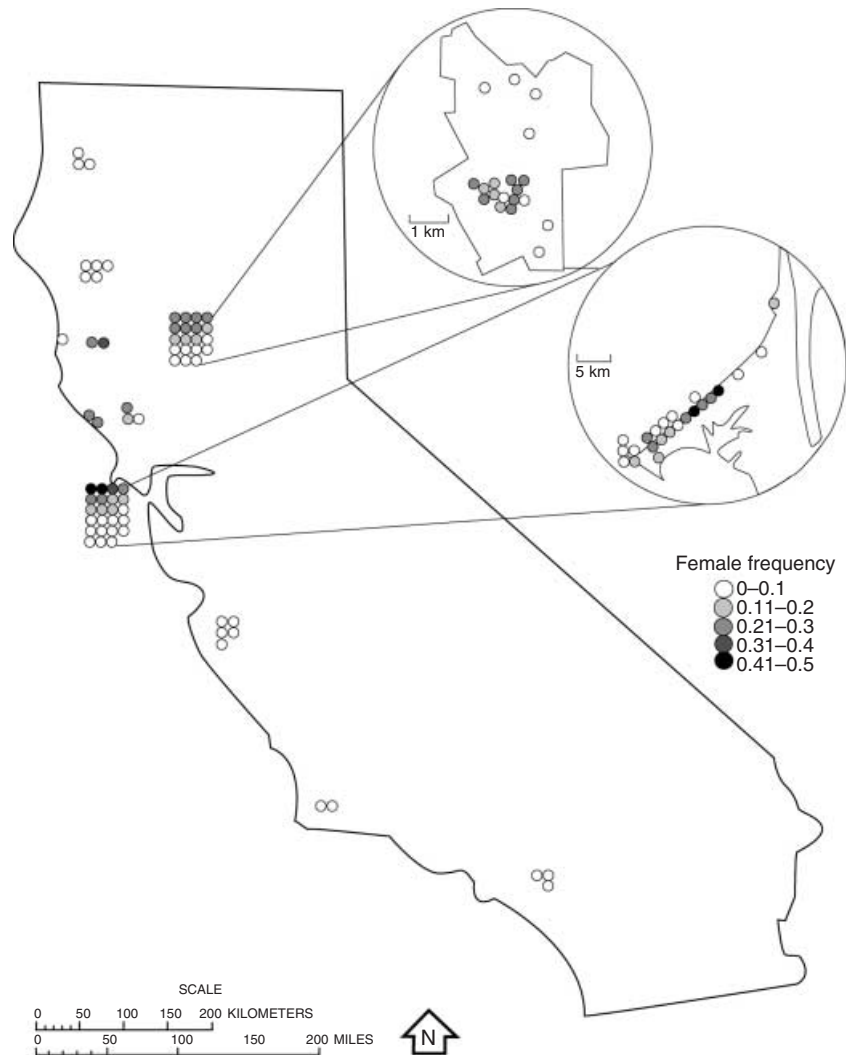
### Sex ratios

Female frequencies up to 0.5 are found in the SNCR, with frequencies  $<0.1$  in the rest of the state (Fig. 3).

**Table 1** Average progeny corolla colours, female frequencies, and progeny sample sizes from greenhouse crosses from which progeny were scored for colour to test for intermediary of hybrid crosses.

Cross	Progeny corolla colour		Progeny female frequency	
	Average $\pm$ var	N	Females (%)	N
White $\times$ White				
CovRM46 $\times$ CovRM11	1 $\pm$ 0	50	0	61
CovRM46 $\times$ CovTT5a	1 $\pm$ 0	22	0	25
TH56 $\times$ TH19	1 $\pm$ 0	24	0	46
TH19 $\times$ TH56			0	19
TH56 $\times$ CovRM46	1 $\pm$ 0	19	0	31
TH56 $\times$ CovRM11	1 $\pm$ 0	13	0	23
TH56 $\times$ CovTT5a			0	11
CovRM46 $\times$ TH19	1 $\pm$ 0	30	0	84
CovRM46 $\times$ TH56			0	79
Average	1.00		0	
Blue $\times$ Blue				
Hop1156 $\times$ Hop1141	4.14 $\pm$ 0.34	29	38	34
Hop1141 $\times$ Hop1156	4.40 $\pm$ 0.36	20	33	24
Average	4.27		36	
White $\times$ Blue				
CovRM11 $\times$ Hop1156	3.58 $\pm$ 0.45	12	55	11
CovRM46 $\times$ Hop1156	3.93 $\pm$ 0.35	29	36	50
CovRM46 $\times$ Hop1141	3.52 $\pm$ 0.81	23	61	57
CovTT5a $\times$ Hop1141			75	16
Average	3.68		57	
Blue $\times$ White				
Hop1156 $\times$ CovRM46	3.66 $\pm$ 0.36	32	100	33
Hop1156 $\times$ TH56	3.00 $\pm$ 0.44	10		
Hop1141 $\times$ CovRM46	3.90 $\pm$ 0.36	31	97	31
Hop1141 $\times$ TH56	2.81 $\pm$ 0.56	16	100	20
Hop1156 $\times$ CovRM11	3.46 $\pm$ 0.35	24	92	24
Hop1156 $\times$ CovTT5a	3.58 $\pm$ 0.81	12	100	14
Hop1141 $\times$ CovRM11	3.36 $\pm$ 0.72	22	100	17
Hop1141 $\times$ CovTT5a	3.18 $\pm$ 0.47	33	100	33
Average	3.37		98	

Individuals were used from four populations: Titlow Hill (TH), CoveloRM (CovRM), CoveloTT (CovTT), and Hopland (Hop). The numbers after each population acronym indicate the individual plant used in the crosses. Maternal parent is listed first and paternal parent second.



**Fig. 3** Map of California showing the location of 65 *Nemophila menziesii* populations and the percentage females in each population.

### Predictions from greenhouse crosses

#### *Within- and between-colour progeny sex ratios*

All crosses between white individuals (five plants), even between Covelo and Titlow Hill, which are separated by over 140 km, produced all hermaphrodite progeny (Table 1), which is the same as found in the natural populations from which these plants came. Crosses between the two blue individuals produced on average 36% females, a value slightly higher than the 20–30% observed in the natural population. Crosses between white  $\times$  blue plants (five plants) produced on average 57% females, and crosses between blue  $\times$  white (six plants) segregated on average 98% females, frequencies which are much higher than those that segregated in the within-colour crosses and also than those found in any of the natural populations. There were significant differences among these four crosses in percentage females segregated ( $X^2_3 = 22.20$ ,

$P < 0.0001$ ). These results indicate that the white individuals selected from three populations possess similar nuclear restorer alleles and possibly similar CMS types. Blue and white plants, on the contrary, appear to have different CMS types, and when hybridized yield high frequencies of females.

#### *Differences in male-sterile cytotypes*

Monte Carlo simulations for two of the four pairs of blue  $\times$  white (and reciprocals) crosses that had at least 10 progeny showed that these crosses and their reciprocals segregate significantly different progeny sex ratios (Table 2). Because there is only partial dependence of data in some of the tests, the significance level adjustment provides for a conservative test, and one other blue  $\times$  white pair of crosses was nearly significantly different (Table 2). These data indicate that Covelo (white) and Hopland (blue) populations carry different male-sterile cytotypes. Two of the four blue  $\times$

**Table 2** Monte Carlo simulation results of female : hermaphrodite progeny sex ratios of 18 pairs of reciprocal crosses.

Cross	P-value
White × white	
TH56 × TH19	1.000
CovRM46 × TH56	1.000
Polymorphic × polymorphic	
NB1009 × NB947	1.000
Blue × blue	
Hop1156 × Hop1141	0.782
White × blue	
CovRM46 × Hop1141	<0.001*
CovRM46 × Hop1156	<0.001*
CovTT5a × Hop1141	0.009
CovRM11 × Hop1156	0.020
Blue × polymorphic	
Hop1156 × NB1009	<0.001*
Hop1156 × NB947	0.002*
Hop1141 × NB1009	0.007
Hop1141 × NB947	0.314
White × polymorphic	
CovTT5a × NB947	1.000
CovRM46 × NB1009	0.024
CovRM46 × NB947	0.502
NB1009 × TH19	0.510
NB947 × TH56	0.516
NB947 × TH19	1.000

Ten thousand randomizations were run with number of female and hermaphrodite progeny for the cross-listed below and for its reciprocal. An asterisk indicates  $P < 0.003$ .

polymorphic crosses had significantly different progeny sex ratios, while none of the seven white × polymorphic crosses had a significantly different progeny sex ratio. These data indicate that the male-sterile cytotypic and/or restorer alleles of the one population I chose as a representative of the polymorphic populations may be more closely allied to white populations than to blue populations. Within-colour reciprocal crosses did not segregate significantly different sex ratios.

#### *Relative ability to produce hybrid seeds*

Almost no between-population fertile seed was made with the two plants from Hastings; the few seeds made (a total of 16 seeds from 407 flowers pollinated) are very likely the result of contamination with self-pollen. All other populations made fertile seed in crosses to each other. There were significant effects on seeds/flower of the corolla colour of both maternal and paternal plants and their interaction (Table 3a). There are no significant difference in seeds per flower between within-colour crosses (mean = 3.54) and between-polymorphic crosses (mean = 4.36) ( $F_{1,72} = 0.06$ ,  $P = 0.81$ ), however, between-polymorphic crosses make more seeds per flower than do blue × white crosses (mean = 2.59) ( $F_{1,74} = 18.11$ ,  $P < 0.0001$ ). The two

**Table 3a** Analysis of relative ability to produce hybrid seed: ANOVA results.

Source	d.f.	SS	F-value	$P > F$ -value
Seeds/flower				
Dam colour	2	154.92	14.14	<0.0001
Sire colour	2	67.48	6.16	0.0029
Dam colour × sire colour	4	71.72	3.27	0.0141
Error	112	613.56		

**Table 3b** Analysis of relative ability to produce hybrid seed: average seeds per flower of crosses based on corolla colour of parents.

	Average seeds/flower ± SE
Blue × blue	4.19 ± 0.15
White × white	3.29 ± 0.67
Blue × white	2.54 ± 0.49
White × blue	2.64 ± 0.48
Blue × polymorphic	4.46 ± 0.80
Polymorphic × blue	6.53 ± 0.76
White × polymorphic	3.28 ± 0.86
Polymorphic × white	4.53 ± 0.44
Polymorphic × polymorphic	7.90 ± 2.94

plants from the polymorphic population made more seeds as maternal parents than any other plant, regardless of the sire (Table 3b).

#### *All-hermaphrodite populations as maternal parents in hybrid crosses*

Progeny from hybrid crosses using three plants from two of the three white populations as maternal parents, CoveloTT and CoveloRM, were on average 57% female (Table 1). This segregation of females indicates the presence of male-sterile cytoplasm that are completely masked by nuclear restorer alleles in the field. Hybrid crosses using plants from Titlow Hill as maternal parents did not set enough seed to generate progeny sex ratios, however, reciprocal crosses between Titlow Hill and Covelo populations do not indicate that these white populations have diverged for male-sterile cytotypic and restorer alleles (although see Discussion for a caveat on this interpretation) (Table 2).

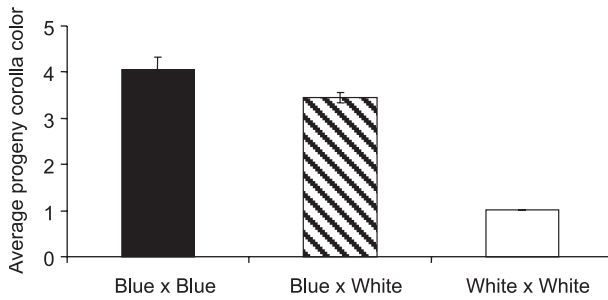
#### **Predictions from field correlations**

##### *Corolla colour inheritance*

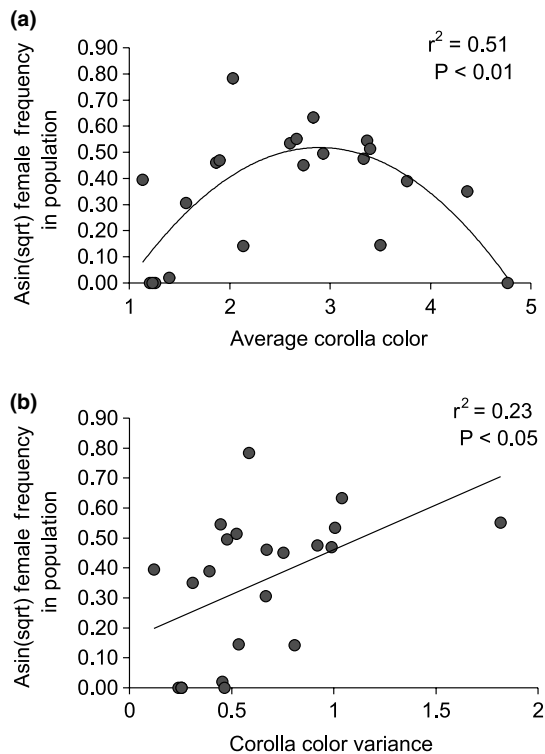
Average corolla colour of progeny from between-colour crosses was intermediate between that from within-colour crosses and there was significant effect because of cross ( $X^2_3 = 15.74$ ,  $P < 0.001$ ) (Fig. 4).

##### *Colour mean and variance regression*

There was a significant quadratic relationship between mean corolla colour and the percentage of females in a



**Fig. 4** Average progeny corolla colour for three types of greenhouse crosses: blue  $\times$  blue, blue  $\times$  white/white  $\times$  blue, and white  $\times$  white. The individual crosses from which the progeny corolla colours were measured are listed in Table 2.



**Fig. 5** (a) Quadratic regression of percentage females in population as a function of the average corolla colour value for each population,  $y = -0.1418x^2 + 0.8203x - 0.6685$ . (b) Linear regression of percentage females in population as a function of the variance in corolla colour for each population,  $y = 0.2987x + 0.1617$ .

population for 22 populations (model  $F_{2,19} = 9.94$ ,  $P < 0.01$ , quadratic term  $t_{1,19} = -4.26$ ,  $P < 0.001$ , linear term  $t_{1,19} = 4.43$ ,  $P < 0.001$ ,  $r^2 = 0.51$ ) (Fig. 5a). There was a significant positive linear relationship between the variance in corolla colour and the percentage of females in a population for 22 populations ( $F_{1,20} = 5.86$ ,  $P < 0.05$ ,  $r^2 = 0.23$ ) (Fig. 5b).

## Discussion

The experimental and observational evidence supports the two main predictions that sex ratio distorters are widespread and associated with corolla colour, and that hybridization of diverged groups has led to the region of high female frequencies.

### Greenhouse crosses

The greenhouse crosses show that crosses between populations of different corolla colour yield much higher frequencies of females than crosses within colour (Table 1). Significant and nearly significant differences in the four reciprocal between-colour crosses show that the plants from these populations have different CMS genes (Table 2). Within-colour crosses showed no significant differences, although this may indicate the mutual presence of restorer alleles for each cytotype and not necessarily identical cytotypes (Koelewijn & van Damme, 1995). These results have two implications: that hybridization of *N. menziesii* between populations with different corolla colours can indeed have an effect on the sex ratio, and that sex ratio distorters may have diverged with corolla colour. It is interesting to note that the blue population used in the crosses has a fairly high percentage females in the field (26–33%), which is unusual for populations with such blue corollas even in the local area in which this population is located (C. M. Barr, unpublished data). This population may have also experienced some hybridization. Although the crosses are insufficient to determine the genetics of restoration, self-crosses of the two Hopland plants segregate *c.* 30% females suggesting heterozygote parents and a recessive restorer (C. M. Barr, unpublished data). However, the asymmetry in females segregated from white  $\times$  blue and blue  $\times$  white crosses is such that a single locus recessive restorer at Hopland is not possible so it seems likely that restoration involves more than one locus.

Within-colour crosses make fewer seeds than do between-colour crosses with the polymorphic population, although this result is not significant. Between-polymorphic crosses make more seeds than blue  $\times$  white crosses. This may indicate that the polymorphic population is a hybrid between blue and white plants and has therefore greater genetic similarity and reduced reproductive isolation from blue and white plants than do blue and white plants from each other. Alternatively, this result may just be a consequence of the fact that plants from the polymorphic population (NB) make more seeds/flower as maternal parents than do any other plants, regardless of sire (Table 3b). However, if the crosses with the polymorphic population as maternal parent are removed, leaving only those with the polymorphic parent as the sire, the mean seed/flower is still larger than that for blue  $\times$  white (3.62 vs. 2.64, respectively,

although this difference is not significant). Plants from the polymorphic populations tend to be vigorous seed producers in greenhouse crosses. It is not clear why this is so, although hybrid vigour is a possibility.

The two Covelo white populations clearly contain a male-sterile cytoplasmic genotype that is completely masked in the wild populations, shown by the segregation of female progeny from white (dam) × blue (sire) crosses. The one successful hybrid cross with the third white population (Titlow Hill) segregated 100% females, and crosses between Titlow Hill and the Covelo populations did not segregate any females, suggesting that Titlow Hill has the same male-sterility genetics as the two Covelo populations. This result is noteworthy because it shows the potential widespread presence of completely masked sex ratio distorters in a natural plant species, confirming expectations from genomic conflict theory (Frank, 1991; Hurst & Pomiankowski, 1991).

### Field correlations

Populations that have intermediate averages and higher variance in corolla colour have higher female frequencies (Fig. 5). In greenhouse crosses, hybrids between individuals from the two corolla colours had colours intermediate to those of crosses within colour (Fig. 4). Corolla colour in hybrids of other species is often intermediate between that of the parents (Stace, 1975; Heywood, 1986; Grant & Wilken, 1988; Klier *et al.*, 1991; but see Rieseberg & Ellstrand, 1993) and hybrid populations are often more polymorphic than their parental populations (Barton & Hewitt, 1985). A similar study on *Limnanthes* also found that populations that contained females were more polymorphic for 12 morphological characters including anther colour, as well as for allozyme markers (Kesseli & Jain, 1987). The data presented here thus suggest that populations with higher female frequencies may be hybrids between populations with all-blue and all-white corollas, and that hybridization may be the cause of the high female frequencies.

There is evidence that the SNCR may be a hybrid zone for many species (B. G. Baldwin and T. M. Hardig, personal communication, 2002). A hybrid population of *Ceanothus* that appeared to involve four parental species has been described in Sonoma County (Howell, 1940; Nobs, 1963; Raven & Axelrod, 1995; but see Hardig *et al.*, 2002). Hybridization in the Bay Area between the oaks *Quercus agrifolia* and *Q. wislizenii* has also been noted (Schoenherr, 1992) and between sympatric species of *Blepharizonia* (Baldwin *et al.*, 2001). It is unclear why this region may facilitate hybridization, however, it may be due to geological movements in this area. The Coast Ranges are separated into two ranges, the Southern Coast Ranges and the Northern Coast Ranges by the San Francisco Bay. Geologically, the two ranges differ, and the Southern Coast Ranges were formed relatively recently to the east and moved to their present location

through right-lateral slip, while the Northern Coast Ranges are older (Schoenherr, 1992). It seems possible that this geological movement brought into contact previously isolated groups of plants.

Hybridization events may help to explain the presence of females in other species. The maintenance of females in hermaphroditic populations has been a troubling issue since Darwin (1877), with populations expected to move to the more stable points of dioecy or back to hermaphroditism. All three main models explaining the maintenance of females in populations with CMS require delicate balances of restorer cost, fitness advantages of male-sterile cytotypes, and gene flow, with populations easily reverting to hermaphroditism (Frank, 1989; Gouyon *et al.*, 1991; Couvet *et al.*, 1998; reviewed in Frank & Barr, 2002). These processes can maintain females over short durations, and may be responsible for preventing fixation of restorers in the presumed hybrid zone of *N. menziesii* after hybridization occurred. However, it seems likely that genetic drift over longer durations would yield fixation of cytotypes and restorers and thus hermaphroditism, especially if CMS types were relatively few. This would help explain the apparent lack of females in most plant species (Yampolsky & Yampolsky, 1922). Rare hybridization events between restored populations may reinitiate conflicts, which then become maintained over relatively shorter durations by the processes described above.

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