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Mating systems of *Cuphea laminuligera* and *Cuphea lutea*

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Summary. In this paper, the mating systems of experimental populations of *C. laminuligera* and *C. lutea* are described. Outcrossing rates (t) were estimated for four populations of *C. laminuligera* and three populations of *C. lutea* using allozyme phenotypes of open-pollinated individual plant families. Populations were grown at densities of 1.0 × 1.0 m (low) and 0.04 × 0.3 m (high). Pollen and ovule frequencies and single locus and multilocus outcrossing rates were estimated for each population using the mixed-mating model. Multilocus estimates of t ranged from 0.83 to 0.98 and 1.00 to 1.01 for low and high density populations of *C. laminuligera,* respectively, and 0.17 to 0.26 and 0.36 to 0.54 for low and high density populations of *C. lutea,* respectively. *C. laminuligera* is predominantly allogamous; however, selfing rates as great as 17% were observed for this species. *C. lutea* is predominantly autogamous, but outcrossing rates as great as 54% were observed for this species. Outcrossing rates increased as density increased within *C. lutea* populations.

Key words: Outcrossing rate – Plant density – Mixed mating model – Isozymes

Introduction

Many species of *Cuphea* are excellent natural sources of medium-chain fatty acids (MCFAs) (Graham et al. 1981; Wolf et al. 1983; Graham 1988, 1989), but few have promise as oilseed crops (Knapp 1990). *C. laminuligera* and *C. lutea* have been investigated to assess their value as new crops (Hirsinger and Knowles 1984; Knapp 1990) and while these species are herbaceous annuals adapted to temperate climates, certain traits impede their domestication (Hirsinger and Knowles 1984; Knapp 1990). The most serious problem is seed shattering. The placenta, with seeds attached, emerges through the capsule and floral tube, leaving maturing seeds exposed and free to dehisce (Graham 1989). Seed dormancy is an other undesirable trait (Hirsinger and Knowles 1984; Graham 1989; Knapp 1990).

The floral morphology of *C. laminuligera, C. lutea,* and several other *Cuphea* species has been described (Graham 1988). *C. laminuligera* and *C. lutea* have two large dorsal petals and four smaller ventral petals attached to a calyx tube that is 11 mm in length in the former species and 8 mm in the latter (Graham 1988). Both species are self-compatible and protandrous, but the anthers of *C. laminuligera* are exserted from the floral tube at anthesis, whereas the anthers of *C. lutea* are inserted in the floral tube at anthesis (Graham 1988). The mating systems of *C. laminuligera* and *C. lutea* have not yet been described. *C. lutea,* however, is autofertile, whereas *C. laminuligera* is not (Hirsinger and Knowles 1984).

Statistical methods for estimating mating system parameters have been developed to exploit codominant molecular markers (Clegg 1980; Ritland and Jain 1981; Shaw et al. 1981; Schoen and Clegg 1986). The mixed mating model is a prominent example. Ritland and Jain (1981) have described maximum likelihood methods for estimating mixed mating model parameters, e.g., outcrossing rates (t) and pollen allele frequencies (p), using individual plant progeny arrays. In this paper we describe experiments which used these methods to estimate outcrossing rates of experimental populations of *C. laminuligera* and *C. lutea.*

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Materials and methods

Estimates of mating system parameters were made in populations of *C. laminuligera* and *C. lutea* derived from reproductively isolated open-pollinated experimental plantings. Plots of a given species were separated by 2 km to ensure reproductive isolation.

The *C. laminuligera* populations used were LA86, LA87, LA11, and LA13. LA11 and LA13 were open-pollinated wild populations. Numerous polymorphic loci with multiple alleles have been observed in these populations (Krueger and Knapp 1990). We derived LA86 and LA87 from LA11 and LA13 by bulking three F_2 populations to maximize the number of polymorphic loci, while minimizing the number of alleles segregating at a given locus.

The *C. lutea* populations used were LU15, LU34, and LU36. LU15 is an F_2 population derived from the cross between parental lines LU06 and LU07. These lines are known to be homozygous for different alleles at three allozyme loci (Krueger and Knapp 1990). LU34 is an open-pollinated population derived from LU15. LU36 was derived by bulking equal amounts of seed of LU06 and LU07.

Two locations in Corvallis, Oregon were used for isolation in 1987 and 1988. These locations were separated by a distance of 2 km. We relied on wild bee populations, mainly bumblebees (*Bombus* sp.), for pollination. *C. laminuligera* and *C. lutea* populations were grown in separate plots at the same location.

Eight-week-old plants were transplanted to the field on May 29, 1987 using a 1×1 m spacing (low density); 60 plants were planted in each plot. Seeds were harvested from individual plants on August 21, 1987 and September 8, 1987. Not more than two seeds per flower were harvested, and several flowers were sampled to eliminate the possibility that all of the seeds collected were the result of a single pollination event or a single pollinator visit.

Bumblebees, the primary pollinators of experimental plantings of *Cuphea* in western Oregon, tend to intensively work individual plants in spaced-plant nurseries; as a consequence, we hypothesized that plant density might significantly affect outcrossing rate. To test this hypothesis, we used low density (1×1 m) and high density (0.04×0.30 m) planting rates. Plots were established by direct seeding on May 10, 1988. Seed was harvested separately from several flowers from each plant on September 20, 1988. We harvested 60 plants from each plot; however, due to poor stand establishment, the low density planting of *C. lutea* at one location was limited to 25 plants.

Seeds of individual plant families were germinated on blotting paper at 26 °C under fluorescent light (12-h day). Germination was nearly 100%. Four-to-seven-day-old cotyledons were electrophoretically assayed as previously described (Knapp and Tagliani 1989). Twenty individuals per family were assayed from populations grown in 1987. Fifteen individuals per family were assayed from populations grown in 1988. A slightly modified AC buffer system (Clayton and Tretiak 1972, Knapp and Tagliani 1989) was used to resolve aconitase (ACO, E.C.4.2.1.3), fluorescent esterase (FES, E.C.3.1.1.1), and shikimate dehydrogenase (SKD, E.C.1.1.1.25) bands in *C. laminuligera* and ACO, 6-phosphogluconic dehydrogenase (PGD, E.C.1.1.1.44) and SKD bands in *C. lutea*. Standard ACO, FES, PGD, and SKD stains were used (Cardy et al. 1983; Marty et al. 1984). The terminology and genetics of these allozymes have been described (Krueger and Knapp 1990).

Single and multilocus (three to five loci per population) outcrossing rates and gene frequencies were estimated using maximum likelihood methods for the mixed mating model (Ritland and Jain 1981). Maternal genotypes were inferred from progeny arrays. Less frequent alleles were combined to form a synthetic allele when more than three alleles were segregating at a locus.

We used 1,000 bootstrap replicates to estimate bias-corrected confidence intervals for outcrossing rates (Efron 1979). Violations of mixed-mating model assumptions were examined by estimating pollen and ovule allele frequencies and expected genotype frequencies. Pollen and ovule allele frequencies were estimated using maximum likelihood methods for the mixed-mating model (Ritland and Jain 1981). Heterozygote deficiency or excess was estimated from observed and expected genotype numbers by subtracting the expected number of heterozygotes from the observed number of heterozygotes and dividing by the expected number of heterozygotes.

Table 1. Single and multilocus mixed-mating model outcrossing rate estimates (t) and 90% bias-corrected percentile bootstrap confidence interval estimates (Interval) for *C. laminuligera*

Year	Population	Density	No of families	Locus[a]	t[b]
1987	LA86	Low	61	*Aco-2*	0.87 (0.77, 0.96)
				Aco-3	0.90 (0.83, 0.97)
				Fes-2	0.97 (0.88, 1.26)
				Skd-2	0.85 (0.75, 0.94)
				Mean	0.90
				Multilocus	0.83 (0.80, 1.03)
1987	LA87	Low	60	*Aco-2*	1.02 (0.97, 1.09)
				Aco-3	1.02 (0.92, 1.13)
				Fes-2	1.09 (1.01, 1.19)
				Skd-2	1.01 (0.93, 1.08)
				Mean	1.04
				Multilocus	0.95 (0.93, 1.03)
1988	LA11	Low	60	*Aco-1*	0.82 (0.68, 0.93)
				Aco-2	1.06 (0.94, 1.13)
				Aco-3	0.87 (0.75, 0.98)
				Skd-2	1.01 (0.91, 1.10)
				Mean	0.94
				Multilocus	0.91 (0.87, 1.04)
1988	LA11	High	60	*Aco-1*	1.02 (0.93, 1.14)
				Aco-2	1.16 (1.06, 1.25)
				Aco-3	0.86 (0.76, 0.94)
				Skd-2	1.08 (1.00, 1.15)
				Mean	1.03
				Multilocus	1.01 (0.99, 1.04)
1988	LA13	Low	56	*Aco-1*	1.03 (0.96, 1.20)
				Aco-2	0.98 (0.92, 1.05)
				Aco-4	0.98 (0.82, 1.11)
				Fes-2	0.87 (0.75, 0.97)
				Skd-2	1.16 (1.08, 1.28)
				Mean	1.00
				Multilocus	0.98 (0.99, 1.05)
1988	LA13	High	60	*Aco-1*	1.00 (0.88, 1.09)
				Aco-2	0.98 (0.83, 1.09)
				Aco-4	0.92 (0.73, 1.05)
				Fes-2	0.91 (0.83, 1.06)
				Skd-2	1.26 (1.19, 1.30)
				Mean	1.01
				Multilocus	1.00 (1.01, 1.08)

[a] Mean is the mean of single locus estimates

[b] Confidence interval estimates are shown in parentheses. The confidence coefficient used was 0.90

Table 2. Observed and expected numbers of single-locus genotypes of *C. laminuligera*

Year	Location	Population	Density	Locus	Genotype						χ^2	P
					11	12	13	22	23	33		
1987	1	LA86	Low	*Aco-2*	46 51.4	218 217.3	84 72.8	350 339.5	209 235.7	63 55.3	6.71	0.17
				Aco-3	53 49.5	291 298.1		627 624.4			0.43	0.51
				Fes-2	645 640.9	295 295.2		30 35.0			0.74	0.39
				Skd-2	318 293.2	164 195.2	265 262.2	44 44.7	87 90.3	88 86.4	7.28	0.12
1987	2	LA87	Low	*Aco-2*	39 44.8	273 271.0	138 122.1	343 331.5	261 291.2	65 58.2	7.16	0.13
				Aco-3	35 44.8	389 366.2		697 710.1			3.80	0.05*
				Fes-2	577 578.0	487 477.6		56 65.0			1.43	0.23
				Skd-2	344 311.4	232 245.3	305 337.1	32 42.6	141 115.4	67 66.1	15.52	<0.01**
1988	1	LA11	Low	*Aco-1*				521 539.7	318 293.6	56 61.7	3.20	0.07
				Aco-2	151 160.4	256 268.8	174 181.0	126 100.3	139 143.4	50 42.1	9.63	0.05*
				Aco-3	69 65.3	293 308.9		493 480.9			1.33	0.25
				Skd-2	54 58.1	210 202.9	152 143.2	168 168.1	234 239.5	76 82.2	1.55	0.82
1988	1	LA11	High	*Aco-1*				566 575.4	294 280.6	28 32.0	1.29	0.26
				Aco-2	172 149.9	239 263.4	181 188.9	88 84.3	165 158.8	42 41.7	6.26	0.18
				Aco-3	85 84.2	317 323.8		466 460.0			0.23	0.63
				Skd-2	33 31.9	188 177.2	106 121.4	170 191.4	315 281.7	74 84.2	10.21	0.04*
1988	2	LA13	Low	*Aco-1*	13 16.5	226 214.5		511 519.0			1.48	0.22
				Aco-2	22 27.8	46 52.5	194 174.0	35 29.3	169 177.0	284 288.0	5.84	0.21
				Aco-4	9 8.2	157 155.2		584 587.2			0.12	0.73
				Fes-2	424 417.8	263 273.7		63 58.5			0.86	0.36
				Skd-2	87 89.1	209 208.2	145 154.3	67 80.1	192 167.8	49 48.7	6.30	0.18
1988	2	LA13	High	*Aco-1*	17 18.9	240 227.7		643 653.4			1.02	0.31
				Aco-2	64 62.0	80 92.6	229 253.5	41 36.0	179 191.5	306 265.2	11.93	0.02*
				Aco-4	26 27.5	243 235.3		618 624.3			0.40	0.53
				Fes-2	506 509.4	338 311.2		58 60.3			0.25	0.62
				Skd-2	86 91.6	189 176.0	280 275.7	26 30.5	200 202.9	116 120.3	2.23	0.69

Expected genotype frequencies were calculated as the binomial or trinomial square of allele frequencies
* $P \leq 0.05$; ** $P \leq 0.01$

Results and discussion

Single-locus outcrossing rate estimates ranged from 0.82 to 1.16 and 0.86 to 1.26 among *C. laminuligera* populations grown at low and high density, respectively (Table 1). Multilocus estimates of t ranged from 0.83 to 0.98 and 1.00 to 1.01 for populations grown at low and high density, respectively.

The effect of plant density on outcrossing rate in *C. laminuligera* was negligible. Outcrossing rates at low plant densities were slightly less than those at high densities; however, they were not significantly different (Table 1).

Single-locus estimates of t for *C. laminuligera* populations occasionally exceeded 1.0 (Table 1). This may have been caused by sampling effects, disassortative mating, heterozygote selection, or by using an inappropriate model (Brown et al. 1985).

Because single-locus estimates of t sample the same mating events, estimates within a population should be equal; however, single-locus outcrossing rate estimates within a population differed by as much as 35% (Table 1). There were significant differences among these estimates (Table 1). This variation could be due to selection acting differentially on loci linked to allozyme marker loci (Brown et al. 1985); however, with the exception of *Skd-2,* which routinely exhibits segregation distortion (Krueger and Knapp 1990), this is an unlikely explanation. Heterogeneity among these estimates may have been caused by different allele frequencies and levels of polymorphism (Ritland and Jain 1981; Brown et al. 1985).

We compared observed and expected genotypic numbers to examine whether or not model assumptions were violated. Observed numbers were not significantly different from expected numbers for most loci (Table 2). Observed frequencies were significantly different for 5 of 26 tests. These differences were mainly limited to *Aco-2* and *Skd-2* (Table 2). Observed numbers of heterozygous individuals among *C. laminuligera* populations were within 5% of expected values; thus, there were no marked heterozygote deficiencies or excesses.

In *C. lutea,* outcrossing rates increased as plant density increased (Table 3). Single-locus estimates of t for *C. lutea* ranged from 0.14 to 0.27 and 0.27 to 0.54 in populations grown at low and high plant density, respectively. Multilocus estimates of t ranged from 0.17 to 0.26 and 0.36 to 0.54 for populations grown at low and high density, respectively.

We tested the *C. lutea* data for goodness of fit to model expectations. Observed and expected genotype frequencies were significantly different for LU36 grown at a low plant density (Table 4). This was probably caused by sampling variation since this population was limited to 25 families (Table 3). Observed and expected genotype numbers did not differ significantly in any other population (Table 4).

Table 3. Single and multilocus mixed-mating model outcrossing rate estimates (t) and 90% bias-corrected percentile bootstrap confidence interval estimates (Interval) for *C. lutea*

Year	Population	Density	No of families	Locus[a]	t[b]
1987	LU15	Low	60	*Aco-1*	0.20 (0.14, 0.27)
				Pgd-1	0.20 (0.16, 0.27)
				Skd-1	0.22 (0.15, 0.37)
				Mean	0.21
				Multilocus	0.21 (0.17, 0.28)
1987	LU15	Low	60	*Aco-1*	0.22 (0.16, 0.29)
				Pgd-1	0.20 (0.13, 0.26)
				Skd-1	0.14 (0.10, 0.19)
				Mean	0.19
				Multilocus	0.17 (0.13, 0.21)
1988	LU34	Low	60	*Aco-1*	0.25 (0.17, 0.32)
				Pgd-1	0.21 (0.15, 0.28)
				Skd-1	0.20 (0.13, 0.28)
				Mean	0.22
				Multilocus	0.22 (0.16, 0.27)
1988	LU34	High	60	*Aco-1*	0.36 (0.29, 0.43)
				Pgd-1	0.43 (0.34, 0.51)
				Skd-1	0.27 (0.21, 0.41)
				Mean	0.35
				Multilocus	0.36 (0.30, 0.42)
1988	LU36	Low	25	*Aco-1*	0.27 (0.23, 0.56)
				Pgd-1	0.27 (0.23, 0.55)
				Skd-1	0.27 (0.23, 0.55)
				Mean	0.27
				Multilocus	0.26 (0.20, 0.52)
1988	LU36	High	50	*Aco-1*	0.50 (0.31, 0.58)
				Pgd-1	0.53 (0.39, 0.60)
				Skd-1	0.54 (0.29, 0.61)
				Mean	0.52
				Multilocus	0.54 (0.38, 0.60)

[a] Mean is the mean of single locus estimates
[b] Confidence interval estimates are shown in parentheses. The confidence coefficient used was 0.90

Protandry and anther exsertion minimize self-fertilization within *C. laminuligera*; however, self-fertilization rates as high as 18% were observed (Table 1). *C. laminuligera* has an absolute requirement for insect pollination. The long (11 mm) floral tube of this species limits honeybee (*Apis* sp.) activity; however, *Bombus* sp. effectively forage within this species (Knapp 1990).

In contrast to *C. laminuligera*, *C. lutea* is autofertile and, prior to this study, was presumed to be highly autogamous. *C. lutea* is protandrous and self-compatible, but its anthers dehisce inside the floral tube (Graham 1988). Although this insures seed set in the absence of insect pollinators, outcrossing rates from 14% to 54% were observed (Table 3). Bumblebees and honeybees actively pollinate *C. lutea* flowers. This is a significant observation since *C. lutea* is one of the few *Cuphea* species active-

Table 4. Observed and expected numbers of single-locus genotypes of *C. lutea*

Year	Location	Population	Density	Locus	Genotype			χ^2	P
					11	12	22		
1987	1	LU15	Low	*Aco-1*	400 393.6	332 338.4	468 468.0	0.23	0.64
				Pgd-1	430 448.4	315 305.7	453 444.8	1.19	0.28
				Skd-1	517 501.2	363 369.3	319 327.3	0.82	0.37
1987	2	LU15	Low	*Aco-1*	426 430.8	372 382.8	402 386.4	0.99	0.32
				Pgd-1	452 440.0	354 366.0	394 391.2	0.56	0.46
				Skd-1	337 334.2	351 349.8	510 512.7	0.04	0.84
1988	1	LU34	Low	*Aco-1*	285 270.2	193 206.8	402 403.0	1.73	0.19
				Pgd-1	322 335.3	219 210.4	358 353.3	0.94	0.33
				Skd-1	299 285.9	249 252.6	351 360.5	0.90	0.34
1988	1	LU34	High	*Aco-1*	234 239.3	271 273.4	398 391.0	0.26	0.61
				Pgd-1	433 432.5	285 287.2	185 184.2	0.02	0.89
				Skd-1	284 279.9	273 264.6	345 358.5	0.84	0.36
1988	2	LU36	Low	*Aco-1*	171 187.5	91 73.7	110 110.9	5.52	0.02*
				Pgd-1	173 187.7	89 73.3	112 112.9	4.52	0.03*
				Skd-1	169 187.4	92 73.3	113 112.9	6.58	0.01**
1988	2	LU36	High	*Aco-1*	428 425.9	168 175.1	145 141.0	0.41	0.52
				Pgd-1	424 423.9	178 180.8	139 136.3	0.10	0.76
				Skd-1	421 420.9	182 185.2	138 134.9	0.13	0.72

Expected genotype frequencies were calculated as the binomial or trinomial square of allele frequencies
* $P \leq 0.05$; ** $P \leq 0.01$

ly pollinated by honeybees. We attribute this to flower size and floral tube length (8 mm).

An inverse relationship between outcrossing rate and plant density is expected if pollinator flight distances are density dependent and related plants are found in close proximity (Levin and Kerster 1974). This phenomenon has been observed in wild populations of *Helianthus annuus* (Ellstrand et al. 1978). By contrast, we did not observe an inverse relationship between density and outcrossing effects. The spatial distribution of genotypes should have been random. Furthermore, the densities used in the experimental plantings were far greater than those needed to produce the effects predicted by Levin and Kerster (1974) and found by Ellstrand et al. (1978) for wild populations.

Vaquero et al. (1989) found outcrossing rates of rye (*Secale cereale* L.) increased as density increased. Self-incompatibility was weakened by decreasing density and led to increased selfing. Although their findings are consistent with ours, the causes are different and, as a conse-

quence, the consistency between *Cuphea* and rye is coincidental. Rye is self-incompatible and wind-pollinated, whereas *Cuphea* is self-compatible and insect-pollinated.

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