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Pollen-Ovule Ratio and Gamete Investment in *Pedicularis* (Orobanchaceae)

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Abstract

The *Pedicularis* species provides ideal materials to study floral evolution because of their substantial flower variation based on a narrow genetic basis, even though they are almost exclusively pollinated by bumblebee. These traits allow us to detect the evolutionary trends of floral parameters without considering genetic background and the difference of pollination vectors. The pollen-ovule ratio is widely used to estimate the pattern of resource investment in two sexual functions in flowering plants. Forty species representing all of the corolla types in *Pedicularis* were used to study pollen-ovule ratio, gamete investment, and their correlations. Results show that pollen-ovule ratio does not differ among both different corolla types and taxonomic groups. It is therefore suggested that pollen-ovule ratio should be a parallel evolution. The correlations between pollen-ovule ratio and pollen size (–), and ovule size (+) can be successfully explained in terms of sex allocation theory. The biological significance of such relationships was also discussed. Additionally, we analyzed the pattern of resource investment into female gamete, which has been somewhat neglected, and found that plants have different patterns of gamete investment between the two sexual functions.

Key words: gamete investment; ovule size; *Pedicularis*; pollen-ovule ratio; pollen size; pollination; sex allocation.

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Pedicularis Linn. has about 700 species in the world, and more than 350 species have been recorded in China (Hong 1983; Yang et al. 1998). The Chinese Himalaya region is regarded as the central place of the origin and evolution of *Pedicularis* and includes species with all kinds of corolla types in the genus (Li 1951). Recent studies based on molecular data tend to consider the genus as a monophylogenetic group (Yang et al. 2003). Although the *Pedicularis* species displays substantial variation in floral design, they are almost of the same breeding system. For example, they are exclusively pollinated by bumblebees (Wang and Li 1998; Macior et al. 2001), and are self-compatibile (Karrenberg and Jensen 2000). Therefore, the *Pedicularis* species provides ideal materials to study floral evolution because the variation in floral traits can be ascribed to evolutionary or ecological factors, rather than considered as effects due to phylogenetics and pollination vectors (Macior 1995).

In plant sex allocation theory, the pollen-ovule ratio has been widely used as an indicator for pollination efficiency, and to detect the pattern of gamete investment between male and female function (Cruden and Miller-Ward 1981; Mione and Anderson 1992; Gallardo et al. 1994). Cruden (1977) first developed the pollen-ovule ratio as a conservative indicator of a pollination system and contended that “the more efficient the transfer of pollen, the lower the pollen-ovule ratio should be”. In another study, Cruden and Miller-Ward (1981) compared several components of a pollination system with a pollen-ovule ratio and reported a negative correlation between pollen-ovule ratio and pollen size, which was attributed to the physiological requirements of pollen-pistil interaction during the postpollination

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process (Cruden and Miller-Ward 1981). Such correlations exist beyond doubt, however, it should be more reasonable in terms of a conceptual framework of sex allocation theory (Charlesworth and Charlesworth 1981; Charnov 1982; Queller 1984; Lloyd 1987; Mione and Anderson 1992; Gallardo et al. 1994; Wyatt et al. 2000).

The objectives of this study are: (i) to determine the patterns of gamete investment into male versus female function at the level of species; (ii) to ascertain the correlations of pollen-ovule ratio and components of gamete investment, and (iii) to provide a valid tool for evaluation of the results obtained.

Results

Mean values (±*SD*) of pollen and ovule number and size, total pollen and ovule volume; and pollen-ovule ratios for the 40 studied *Pedicularis* species are all summarized in Table 1. For each variable, the sample size is also shown in Table 1. Table 2 indicates the correlations of parameter means among all species.

Pollen-ovule ratios in *Pedicularis* species vary almost between 1 000 and 7 000. The highest values have been found in *P. rex* C. B. Clarke ssp. *rex* and *P. rex* C. B. Clarke ssp. *lipskyana* (Bonati) P. C. Tsoong ((11 222.04 ± 4 887.18), and (15 153 ± 3 900.89), respectively), and the lowest ones have been found in *P. anas* Maxim. and *P. urceolata* P. C. Tsoong ((425.73 ± 49.80), and (704.41 ± 196.39), respectively). Results indicate that pollen-ovule ratios of species among the four essential corolla types show no significant difference ($F = 2.53$, $P = 0.07$). In addition, analysis based on data from species of five groups (sample size more than five) also does not show any significant difference ($F = 0.71$, $P = 0.59$). Correlation analysis indicates that pollen-ovule ratio correlates with pollen volume ($r = 0.351$, $P = 0.02$) and not with ovule volume ($r = 0.291$, $P = 0.06$). Results also show that there exist significant correlations between both pollen-ovule ratio and pollen size, and pollen-ovule ratio and ovule size ($r = -0.458$, $P = 0.002$; $r = 0.542$, $P = 0.000\ 1$, respectively).

To compare the potential different patterns of resource investment to pollen and ovule at the level of a single flower in *Pedicularis*, we evaluated the contribution of size and the number of gametes to total resource investment per flower. Results show that pollen volume correlates significantly with pollen production ($r = 0.743$, $P < 0.000\ 1$), not significantly with pollen size ($r = 0.053$, $P = 0.73$); while ovule volume correlates significantly with both ovule number and size ($r = 0.655$, $P < 0.000\ 1$; $r = 0.507$, $P = 0.000\ 4$, respectively). However, pollen volume correlates significantly with ovule production ($r = 0.707$, $P < 0.000\ 1$). Not like the case in pollen production, there is a lack of negative correlation between ovule size and ovule number ($r = -0.063$, $P = 0.68$). Coefficient of variation (CV) analysis shows that the ovule number displays substantial variation within both species and genus, that the mean CV value within a species equals 12.59, and that the CV value within the genus (data from the 40 studied species) equals 67.15.

Discussion

In a former study, we reported an interspecific pollen size-number trade-off and positive relationship between pollen and ovule production in *Pedicularis*. These results suggest that the *Pedicularis* species evolves to find a compromise of gamete investment into the two sexual functions and reproductive units to achieve maximum reproductive success (Yang and Guo 2004).

Pollen-ovule ratio in *Pedicularis*

Pollen-ovule ratios are widely used in studying and evaluating plant breeding systems (Cruden 1977; Preston 1986; Ritland and Ritland 1989; Nieto-Feliner 1991; Mione and Anderson 1992; Yashiro et al. 1999; Huang et al. 2002; Jürgens et al. 2002; Damgaard and Abbott 1995; Wyatt et al. 2000). However, sex allocation theories tend to explain pollen-ovule ratios more directly as a result of local mate competition, with allocation ratios in hermaphrodite plants being driven by male/female gain curves for fitness (Charnov 1982). Pollen-ovule ratios in the *Pedicularis* species show substantial variations from 425.73 for *P. anas* Maxim. to 15 153 for *P. rex* C. B. Clarke ssp. *lipskyana* (Bonati) P. C. Tsoong, which may be a reflex of the difference in detailed mating system components for different species. Chernov (1985) argued that *Pedicularis* in Russia was changing from an entomophilous genus to a self-pollinating genus because of a lack of pollinators and that the flowers of the *Pedicularis* species have not yet had time to lose their more advanced characteristics of entomophily. Sun et al. (2005) also reported that *P. dunniana* can accomplish autonomous self-pollination due to corolla wilting when pollinators are absent. To detect the exact reason for abnormal pollen-ovule ratio value in *Pedicularis*, detailed studies on mating system for certain species are urgently needed.

Studies indicate that pollen-ovule ratio is not a phylogenetic indicator, but parallel evolution (Mione and Anderson 1992; Wyatt et al. 2000). In *Pedicularis*, pollen-ovule ratios show no significant difference both in species with different corolla types and in species belonging to different groups. We therefore suggest that trends in pollen-ovule ratios in *Pedicularis* do not appear to reflect taxonomic affinities, but rather ecogeographical pressures, and must result from parallel evolution (Wyatt et al. 2000; Yang et al. 2003). This supports the argument that variation of floral design in *Pedicularis* must be a result of parallel

Table 1. Floral parameters of 40 *Pedicularis* species (mean ± *SD*)

Species	Pollen production[†] *n* > 10	Ovule production[†] *n* > 25	Pollen-ovule ratio	Pollen size[†] (μm^3) *n* > 80	Ovule size (10^{-3} mm^3) *n* > 30	Pollen volume (mm^3)	Ovule volume (mm^3)
Pedicularis anas Maxim.	14 190±2364	34.94±5.00	425.73±49.80	8732.77±591.44	8.65±2.01	0.12±0.004	0.29±0.04
P. axillaries Franch.	31 713±2847	8.17±1.40	3945.27±466.55	6304.34±532.13	19.36±4.45	0.20±0.02	0.16±0.03
P. batangensis Bur. et Franch.	41 428±5787	13.21±1.53	3157.28±444.89	3321.77±646.05	8.19±2.22	0.14±0.02	0.11±0.01
P. confertiflora Prain	36 423±4885	43.58±5.71	843.41±69.57	3982.17±483.73	8.55±2.10	0.15±0.008	0.37±0.05
P. cyathophylla Franch.	84 750±9623	33±3.54	2622.14±388.02	8390.55±439.46	29.63±5.97	0.71±0.05	0.98±0.10
P. cymbalaria Bonati	84 907±6759	34.58±2.97	2469.71±221.61	3091.93±233.58	11.73±2.74	0.26±0.01	0.41±0.03
P. davidii Franch.	33 511±4111	19.23±3.19	1777.85±264.90	7735.95±789.42	33.23±13.90	0.26±0.02	0.64±0.11
P. debilis Franch.	38 228±6194	25.29±5.06	1530.79±150.49	4388.19±388.74	9.31±2.39	0.17±0.03	0.24±0.25
P. densispica Franch.	124 786±23 981	39.42±4.78	3171.53±525.53	1008.35±95.45	7.80±0.5	0.13±0.02	0.31±0.04
P. dichotoma Bonati	40 489±5455	31.31±2.29	1301.57±169.84	2679.63±352.37	16.43±3.78	0.11±0.01	0.51±0.04
P. dolichocymba Hand.- Mazz.	138 393±32 202	66.42±10.03	2113.39±279.26	6166.47±1351.9	37.65±6.27	0.85±0.07	2.50±0.38
P. dolichoglossa H. L. Li	20 020±3568	19.92±2.63	1021.59±140.66	8132.55±537.57	15.79±3.29	0.16±0.006	0.31±0.04
P. dunniana Bonati	136 510±19 921	95.23±5.78	1449.68±128.79	6117.99±821.92	55.09±6.35	0.84±0.06	5.25±0.32
P. elwesii Hook. f.	246 693±35 124	27.83±2.29	8945.06±1190.21	1871.49±274.04	43.34±9.92	0.46±0.03	1.21±0.10
P. geosiphon H. Smith et P. C. Tsoong	17 467±3674	8.71±1.33	1977.32±207.4	8656.31±664.79	44.34±8.85	0.15±0.01	0.40±0.05
P. gracilicaulis H. L. Li	71 694±10 242	32.14±5.43	2257.77±316.70	3035.53±269.81	18.81±4.64	0.22±0.03	0.60±0.10
P. gruina Franch.	59 351±7177	21.12±2.18	2842.06±391.43	4091.61±330.81	27.02±7.58	0.24±0.02	0.57±0.06
P. integrifolia Hook. f.	362 053±25 303	66.00±9.37	5421.22±723.18	2691.61±206.11	12.40±4.43	0.97±0.02	0.82±0.12
P. kansuensis Maxim.	43 468±5462	6.62±0.77	6453.55±924.65	2212.74±255.60	44.72±12.98	0.10±0.009	0.30±0.03
P. lachnoglossa Hook. f.	37 382±4675	13.54±1.76	2806.95±397.71	4076.11±560.59	34.50±6.16	0.15±0.005	0.47±0.06
P. latituba Bonati	26 253±4221	12.46±1.71	2127.73±239.97	9014.65±858.97	22.66±8.52	0.24±0.02	0.28±0.24
P. likiangensis Franch.	26 069±2967	10.80±1.37	2433.21±285.76	2409.30±189.91	9.74±2.84	0.063±0.007	0.11±0.01
P. longiflora Rudolph. var. *longiflora*	22 688±3074	30.64±2.76	746.34±93.49	17 314.44±1372.42	18.75±5.73	0.39±0.03	0.57±0.05
P. longiflora Rudolph. var. *tubiformis* (Koltz.) P. C. Tsoong	22 717±2641	23.08±1.93	992.59±156.82	12 193.76±1361.30	16.93±6.61	0.28±0.03	0.39±0.03

Table 1 (continued)

P. macrosiphon Franch.	16 693±2182	10.00±0.74	1678.35±139.53	10 339.43±773.60	48.13±11.75	0.17±0.005	0.48±0.04
P. megalochila H. L. Li	57 256±10 819	30.10±1.79	1908.58±372.49	4526.10±520.49	24.56±7.09	0.26±0.05	0.74±0.04
P. monbeigiana Bonati	36 697±4755	7.83±0.58	4704.17±409.06	3420.30±457.64	25.69±6.91	0.13±0.009	0.20±0.01
P. mussoti Franch.	49 103±4739	11.15±1.14	4363.78±414.31	6666.73±594.67	49.17±10.94	0.33±0.009	0.55±0.06
P. polyodonta H. L. Li	23 007±5593	15.47±1.92	1527.05±188.79	6994.10±615.68	37.82±7.25	0.16±0.02	0.58±0.07
P. princeps Bur. et Franch.	145 027±23 965	62.80±8.50	2391.32±367.28	5189.95±695.30	20.07±3.73	0.75±0.08	1.26±0.17
P. rex C. B. Clarke ssp. *lipskyana* (Bonati) P. C. Tsoong	334 516±35 898	22.92±5.98	15 153.60±3900.89	2263.96±191.14	117.99±19.76	0.76±0.03	2.70±0.71
P. rex C. B. Clarke ssp. *rex*	330 536±33 356	29.47±1.81	11 222.04±4887.18	2119.81±221.59	88.72±33.33	0.70±0.02	2.61±0.16
P. rhinanthoides Schrenk ex Fisch. et Mey. ssp. *labellata* (Jacq.) P. C. Tsoong	115 543±11 280	26.63±3.24	4374.16±470.56	5903.07±616.41	18.93±4.23	0.68±0.07	0.50±0.06
P. rhinanthoides Schrenk ex Fisch. et Mey. ssp. *rhinanthoides*	124 736±21 359	28.54±2.63	4436.38±610.42	4161.79±392.28	14.77±4.53	0.52±0.03	0.42±0.04
P. rhodotricha Maxim.	113 417±15 870	72.08±7.53	1589.07±194.67	3690.65±426.99	18.08±4.38	0.42±0.03	1.30±0.14
P. roylei Maxim.	42 514±13 661	17.15±1.91	2439.75±493.64	1236.25±141.39	7.06±1.76	0.053±0.017	0.12±0.01
P. siphonantha Don	23 007±3092	15.47±1.92	1527.05±188.79	6994.10±615.68	37.82±7.25	0.16±0.02	0.58±0.07
P. stenocorys Franch.	126 557±21 207	33.58±3.50	3797.23±353.59	3950.44±327.67	14.86±6.22	0.50±0.03	0.50±0.05
P. szetschuanica Maxim.	81 657±11 309	12.33±2.15	6756.59±900.97	1098.32±110.13	7.44±1.85	0.09±0.005	0.09±0.02
P. tenera H. L. Li	89 383±14 829	45.50±6.86	2015.67±443.71	1286.19±191.58	6.58±1.87	0.11±0.01	0.30±0.05
P. trichoglossa Hook. f.	182 052±30 590	66.40±13.15	2832.72±549.12	3784.90±411.76	26.04±4.42	0.69±0.04	1.73±0.34
P. tricolor Hand.-Mazz. ssp. *tricolor*	67 353±7095	25.29±4.16	2751.06±537.19	9154.84±564.20	43.95±13.59	0.62±0.03	1.11±0.18
P. tricolor Hand.-Mazz. ssp. *lophocentra* H. L. Li	64 570±11 861	16.00±2.89	4040.94±365.25	4831.94±843.68	44.29±10.32	0.31±0.06	0.71±0.13
P. urceolata P. C. Tsoong	20 393±2935	27.75±3.99	740.41±196.39	7533.36±664.13	101.08±2.50	0.15±0.02	0.28±0.04

†Data from a former study; see page 179 in Yang and Guo (2004).

Table 2. Correlations among all species on parameter means

	O	P/O	PS	OS	PV	OV
P	**	****	**	*	****	****
	0.446	0.737	–0.424	0.373	0.743	0.547
O		ns	ns	ns	****	****
		–0.169	–0.084	–0.063	0.636	0.655
P/O			**	****	*	ns*
			–0.458	0.542	0.351	0.291
PS				ns	ns	ns
				0.062	0.053	–0.029
OS					*	***
					0.315	0.507
PV						****
						0.708

O, ovules production; OS, ovule size; OV, ovule volume; P, pollen production; P/O, pollen-ovule ratio; PS, pollen size; PV, pollen volume. **** $P < 0.000\ 1$; *** $0.000\ 1 < P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; ns* $0.05 < P < 0.095$; ns, not significant $P > 0.095$; all sample sizes $n = 44$.

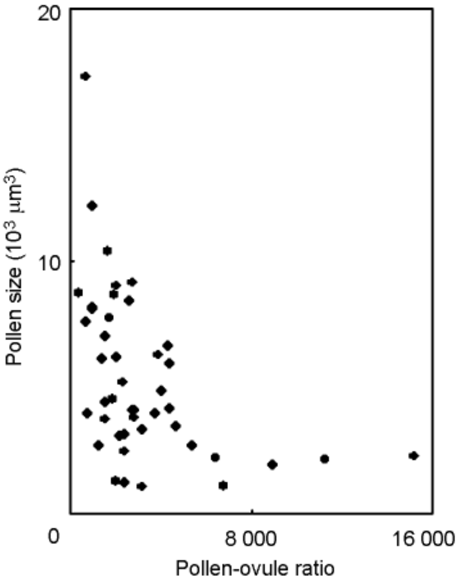

Figure 1. Correlation analysis between pollen-ovule ratio and pollen size ($r = –0.458$; $P < 0.01$).

evolution based on molecular data analysis by Yang et al. (2003).

Pollen-ovule ratio and gamete investment in *Pedicularis*

We found a significant correlation between pollen-ovule ratio and pollen volume per flower (Table 2). Similar to our work, a more significant correlation ($r = 0.699$, $P < 0.001$) was reported by Mione and Anderson (1992) in species of *Solanum* section *Basarthrum*. In addition, our study did not find a significant relationship between pollen-ovule ratio and the total ovule volume per ovary (Table 2). All the results fit in well with the theory of sex allocation (Charnov 1982), in which pollen-ovule ratio is considered to be determined as follows:

$$\log(P/O) = \log[r/(1–r)] + \log C_2 – \log C_1 \quad (1)$$

where r/(1–r) is the allocation of resources between the male function and the female function, and C_2 and C_1 represent the cost in resource units of each ovule and each pollen grain. The equation can explain either the presence or absence of the correlation between pollen-ovule ratio and sex allocation because the variables C_1 and C_2 may be different in different taxa (Gallardo et al. 1994).

It was predicted that pollen-ovule ratio should be inversely proportional to pollen size within a given taxa and breeding system (Cruden and Miller-Ward 1981). The relationship between these two parameters in our results (Figure 1) supports this prediction. The correlation was explained by Cruden and Miller-Ward (1981) that it should be attributed to the physiological requirements of pollen-pistil interaction during the postpollination process. However, some recent studies tend to explain this relationship in terms of sex allocation theory (Mione and Anderson 1992; Gallardo et al. 1994). Equation (1) suggests that a negative correlation between pollen-ovule ratio and pollen size should exist when the other two factors in the equation (as may be the case within a given compatibility-group and breeding system) remain constant (Mione and Anderson 1992; Gallardo et al. 1994). It is then reasonable to find such a correlation in this study since *Pedicularis* species are almost of the same breeding system. For example, *Pedicularis* are exclusively pollinated by bumblebees (Macior et al. 2001) and are self-compatible (Karrenberg and Jensen 2000). According to Equation (1), and considered the similar breeding system of our studied species, there also should exist a positive correlation between pollen-ovule ratio and ovule size. Our results fit in well with this prediction (Figure 2).

Reviewing the evolution of pollen size may provide new insight to understand the negative relation between pollen-ovule ratio and pollen size. Studies have shown that pollen size evolution was influenced strongly by the post-pollination process, natural selection on pollen size results from the requirement of successful fertilization (Harder 1998; Torres 2000; Aquilar et al. 2002). In another study on *Pedicularis*, we found there existed positive correlation between pollination efficiency and pollen size (Yang et al. 2002). It suggests that as pollination efficiency increases, plants evolve to distribute limited resources per reproductive unit, rather than the number of reproductive units (Vonhof and Harder 1995). When a transfer of pollen is inefficient (sometimes a case of higher pollen-ovule ratio), a higher pollen production is needed for reproductive assurance; and a smaller size of pollen grain means a more

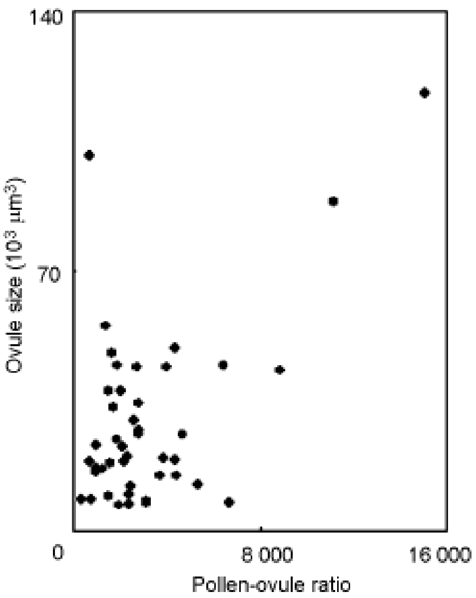

Figure 2. Correlation analysis between pollen-ovule ratio and ovule size (r = 0.542; P < 0.000 1).

efficient pattern of resource investment. A larger size of pollen grain could mean greater wastage of resources if the same number of pollen grains fails to be transferred to the stigma. When pollination efficiency is high (sometimes the case of lower pollen-ovule ratio), loss of resources via the superfluous pollen grains is not high and hence resource investment to increase the size of individual pollen can promote reproductive quality.

Gamete investment into reproductive units

Our results that pollen volume correlates significantly with pollen production (positive), and not significantly with pollen size (Table 2) support the conclusions of our former study that an interspecific pollen size-number trade-off was found and in addition, pollen size varies considerably less than pollen production (Yang and Guo 2004). A similar result has also been reported by other studies (Cruden and Miller-Ward 1981; Mione and Anderson 1992). It was then suggested that pollen grain number alone serves adequately as an indicator of allocation to pollen (Mione and Anderson 1992). In other words, an increased investment to pollen is more manifest as a larger number of pollen grains (Cruden and Miller-Ward 1981). We therefore suggest, in the evolutionary process, that floral trait of pollen grain number per flower is more flexible than pollen size under certain natural selection, and pollen size may be the primary target of natural selection (Vonhof and Harder 1995; Harder 1998; Sarkissian and Harder 2001).

The pattern of resource investment to female gametes is somewhat neglected compared with that of male gametes. Results of this study show a different pattern of resource investment between male and female gametes. Ovule volume significantly correlated positively with both ovule production and ovule size (Table 2). Such a pattern may suggest that when resource investment to ovule increased, a larger size and number of ovules to be fertilized can increase female fitness more effectively than any other pattern, such as increasing ovule number or ovule size alone.

Though several studies have shown that ovule number per ovary tends to be relatively constant (Cruden 1977; Preston 1986; Philbrick and Anderson 1987), the *Pedicularis* species in this study displays high intra and interspecific variations in ovule production. Given that ovule production is related with the breeding system and that higher ovule production within a taxa is frequently associated with naturally higher selfing rates (Schoen 1982; Mione and Anderson 1992), further studies into the breeding system in *Pedicularis* species are needed to explain the observed variations in ovule production.

Materials and Methods

A total of 40 *Pedicularis* species (Table 1) representing all corolla types were collected from the native habitat from 2000 to 2002. All species were found in the Northwest of Yunnan Province and west of Sichuan Province in Southwest China. Voucher specimens are preserved in the Wuhan University Herbarium (WH), China.

According to Li (1951), four essential types of corolla structure are described in *Pedicularis*; (i) toothless with a short flower tube; (ii) toothed with a short flower tube; (iii) beaked with a short flower tube; and (iv) beaked with a long flower tube. Among the four types, the toothless and the toothed forms have been interpreted to be more archaic, while the beaked and long-tubed types are derived (Li 1951; Yang et al. 2002). In Tsoong's taxonomic system, *Pedicularis* species were divided into 13 groups (Tsoong 1955, 1956, 1961). Yang et al. (2002) demonstrated that *Pedicularis* species have different pollination efficiency because of the variations in stigmatic surface.

With regard to sex allocation, we collected data on the volume of single pollen grain (pollen size), volume of single ovule (ovule size), and pollen and ovule production per flower for the 40 species of *Pedicularis* studied. As a more precise indicator to evaluate resource investment to the two sexual functions (Cruden and Miller-Ward 1981; Mione and Anderson 1992), total pollen and ovule volume per flower (pollen volume and ovule volume, respectively) were also measured. Data about pollen size and pollen production were from our former study (Yang and Guo 2004).

Mature fully-opened flowers for further study in the laboratory were picked randomly during the peak blooming period in each species and quickly fixed in FAA solution constituted of formalin (37%–40%), acetic acid and alcohol (50%) at a ratio of 5:6:89 by volume. Mature flower with undehisced anther were used to measure pollen production. The ovary was carefully dissected out of each flower and placed in a drop of water on a microscope slide. The entire placenta with attached ovules was removed via a longitudinal slit in the ovary wall. The ovules were carefully loosened from the placenta and spread in the drop of water to be counted at 40× magnification under a dissecting microscope. Pollen-ovule ratios were determined by the number of pollen grains divided by the number of ovules produced in a flower.

Ovules were carefully dissected at 40× magnification under a dissecting microscope from mature but undehisced flowers for measurement of ovule size. The shape of ovule in *Pedicularis* is also similar to ellipsoid. Ovule size was estimated by the formula $PE^2/6$, where P is the polar axis diameter and E is the equatorial axis diameter. Pollen and ovule volume were calculated by multiplying the mean pollen grain volume and the mean volume of a single ovule for each flower by the mean pollen and ovule numbers respectively for the flower (Cruden and Miller-Ward 1981; Mione and Anderson 1992).

All statistical tests were performed using the SAS program (SAS Institute 1998). Correlation analysis and tests of significance were used to determine relationships between all variables for all the study species. ANOVA analysis was used to determine the significance of difference in pollen-ovule ratios among species with different corolla types or groups. The variation of ovule numbers within species and genus was treated by CV analysis.

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