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GENETIC DIVERSITY, CLONAL DIVERSITY AND FINE-SCALE SPATIAL GENETIC STRUCTURE OF THE WHITE AND RED FRUIT COLOR MORPHS OF *FRAGARIA PENTAPHYLLA* POPULATIONS

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Abstract

Environmental heterogeneity and biological characteristics of plants may have an impact on the fine-scale spatial genetic structure (FSGS) of plant populations. It is not yet known how fruit color polymorphisms and environmental heterogeneity influence the FSGS. In this study, the genetic diversity, clonal diversity and FSGS of the white and red morphs of the wild strawberry (*Fragaria pentaphylla*) populations at three sites in Mou County, Sichun Province, were analyzed using the simple sequence repeat technique. Among 136, 101, and 141 red morph individuals, 43, 53 and 65 genets were identified; whereas among 18, 22, and 14 white morph individuals, 9, 20, and 12 genets were identified. Genetic diversity were similar between the two morphs of *F. pentaphylla*, however the red morph had higher clonal diversity than the white morph. No significant difference in genetic diversity of th two morphs between populations at different sites. Clonal diversity varied, being largest at site 2, and lowest at site 1. The red morph showed stronger FSGS than white morph. The *x*-intercepts (index indicated the distance that clones can reach) of the red morph were larger than those of the white morph. The FSGS of *F. pentaphylla* varied between the three sites, which could be due to the different investment in vegetative growth influenced by habitats heterogeneity. Our findings may be fundamental for the study of the mechanisms underlying the adaptation strategies of wild strawberries with different fruit coloration.

Key words: *Fragaria pentaphylla*, Genetic variation, Clonal diversity, FSGS, Fruit color, Simple sequence repeats.

Introduction

Fine-scale spatial genetic structure (FSGS), i.e., the non-random spatial distribution of genotypes (Vekemans & Hardy, 2004; Kettle *et al*., 2011), has a significant impact on population biology (Hardy, 2003; Raabová *et al*., 2015; Mazal *et al*., 2021). FSGS can affect the gene flow, effective population size and patterns of viability selection within a plant population (Lopez-Gallego & O'Neil, 2010; Mazal *et al*., 2021) and is one of the key factors in understanding the important factors and processes of maintaining a viable population, particularly those related to gene flow (Escudero *et al*., 2003; Ishihama *et al*., 2005).

Fruits are often brightly colored and fruit color polymorphisms are common and widespread in plants, occurring in at least 19 families of plants (Wilson *et al*., 1989; Whitney & Lister, 2004; Burns, 2005; Chen *et al*., 2020). Although previous studies have demonstrated the pleiotropic effects of fruit colors on the plant reproduction and progeny fitness (Traveset & Willson, 1998; Bach & Kelly, 2007; Messaoud & Boussaid, 2011; Burns, 2015; Chen *et al*., 2020), the ecological and evolutionary significance of fruit color polymorphism remains poorly understood (Whitney & Lister, 2004). Up to now, very little has been known about the effect of fruit color polymorphisms on the FSGS of plant populations.

Both vegetative growth and sexual reproduction could influence the FSGS of plant populations (Gigant *et al*., 2016; Roberta,2020). Clonal reproduction can affect plant FSGS by increasing local genetic drift and geitonogamy (Heywood, 1991; Charpentier, 2001). Clone diversity refers to the level of quantity of genets in a cloned plant population, which can reflect the degree of sexual reproduction of the cloned population. Sexual reproduction could influence the FSGS within plant populations through pollen transfer, seed dispersal and recruitment, genetic drift and gene flow (Gigant *et al*., 2016). It is clear that the fruit color polymorphisms can affect the characterisitcs and fitness of the offspring produced by sexual reproduction, including seed germination rate, and seed dormancy, seedling survival rate and growth. For example, the seeds of the white fruits of *Myrtus communis* are smaller, lighter and more numerous than those of dark blue fruits (Messaoud & Boussaid, 2011). the progeny of orange fruits of the mistletoe *Alepis flavida* had higher survival rates, growth rates and flowering rates than those of red fruits (Bach & Kelly, 2007); The seed germination time of red fruits is approximately 2 days earlier, and the germination rate is 40% higher than that of white fruits. (Chen *et al*., 2020). Moreover, the fruit color polymorphisms can also affect the vegetative growth of plants. Chen *et al*., (2020) found that the offspring of *Fragaria pentaphylla* with red fruit produced 10% more stolons than the offspring of *Fragaria pentaphylla* with white fruit. FSGS is determined by the effects of dispersal (Hardy & Vekemans, 1999; Angbonda *et al*., 2021), especially seed dispersal (Eduardo *et al*., 2008; Araújo *et al*., 2017). The agents that directly influence seed dispersal, including gravity, water, wind and animals, have a significant effect on the FSGS of plant populations (Guariguata & Pinard, 1998). Previous studies have also shown that fruit color polymorphisms would have an effect on animal mediated seed dispersal, due to color preference or conspicuity (Gervais *et al*., 1999; Willson & O'Dowd, 1989; Whitney & Lister, 2004). Therefore, we hypothesized that fruit color polymorphisms influence the sexual and vegetative reproduction, seed dispersal patterns, and consequently affect genetic diversity, clonal diversity and the FSGS of plant populations.

Another important factor affecting the FSGS of plant populations is the environmental heterogeneity (Chung *et al*., 2004; Chung & Chung, 2004), such as altitudinal gradients (Torroba-Balmori *et al*., 2017). Fruit color could also be the result of adaptation to abiotic environments, as well as the products of natural selection under selection pressure from birds, insects or animals (Burns, 2015). Heterogeneity in the environment, such as altitude, can have an effect on the population density of the different fruit color polymorphisms (Gervais *et al*., 1999). Vekemans & Hardy (2003) found that lower-density populations have strong FSGS. So can the environmental heterogeneity directly or indirectly affect FSGS in color morphs directly or indirectly? The answer is still unknown.

Fruit color is an important quality trait of strawberries and influences human consumption (Duan *et al*., 2017). *Fragaria pentaphylla* is a wild strawberry belonging to the genus *Fragraia* in family Rosaceae (Yu, 1974). *Fragaria pentahylla* produces red or white fruits (Bai, 2017; Duan *et al*., 2017). White and red fruits of *F. pentaphylla* have been demonstrated to have different chemical components (Duan *et al*., 2017; Shen *et al*., 2022) and fruits quality (Duan *et al*., 2020). Based on the data from one typicl population of *F. pentaphylla*, the red morph showed stronger clonal growth ability and fitness of seeds than those of white morph (Chen *et al*., 2020). In this study, we compared the genetic diversity, clonal diversity and patterns of FSGS of the white and red fruit color morphs of *F. pentaphylla* at three different sites and aimed to determine whether: 1) the red morph has a higher genetic diversity, higher clonal diversity and stronger FSGS than the white morph, and 2) the FSGS of both two morphs of *F. pentaphylla* are affected by environmental heterogeneity.

Material and Methods

Study species: *Fragaria pentaphylla*, a perennial herb with fruits that occur in white and red color morphs (Duan et al., 2017), is hermaphroditic but self-incompatible (Liston *et al*., 2014), with a sexual reproductive mode via seeds and asexual reproductive mode by producing vegetative progeny, or ramets along stolons (Chen *et al*., 2020). *Fragaria pentaphylla* is a diploid species with 7 chromosome pairs (2n=2X=14) that is widely distributed in various habitats, including montane grasslands, forests, forest clearings, bare, and shrublands, gravelly areas at altitudes ranging from 700 m to 2300 m in southwest China (Hou *et al*., 2018; Yu 1974). Flowering occurs between April and May, and fruit ripens between May and June. The flowers of *Fragaria pentaphylla* were observed visited by ants and small bees (including some species of Megachilidae, Apidae, Andrenidae, and Halictidae), and flies (Syrphidae & Bombyliidae) (Ashman, 2000).

Sample collection: Fresh leaves of *F. pentaphylla*, consisting of both the white and red morphs, were collected in June 2016, from three natural populations on Mountain Hou (Fig. 1), Mao County, Sichuan Province, China. The environmental and anthropogenic characteristics of the three locations are listed in Table 1. To investigate the FSGS of *F. pentaphylla* white and red morphs in the three patches, contiguous quadrats of 20 by 20 cm were established and the spatial coordinates (x, y) were recorded (Figs. 2-3). The youngest fully expanded leaves of the ramets located at the coordinates were sampled and fruit color was recorded. The leaves were put into a plastic self-sealing bag filled with some silica gel immediately.

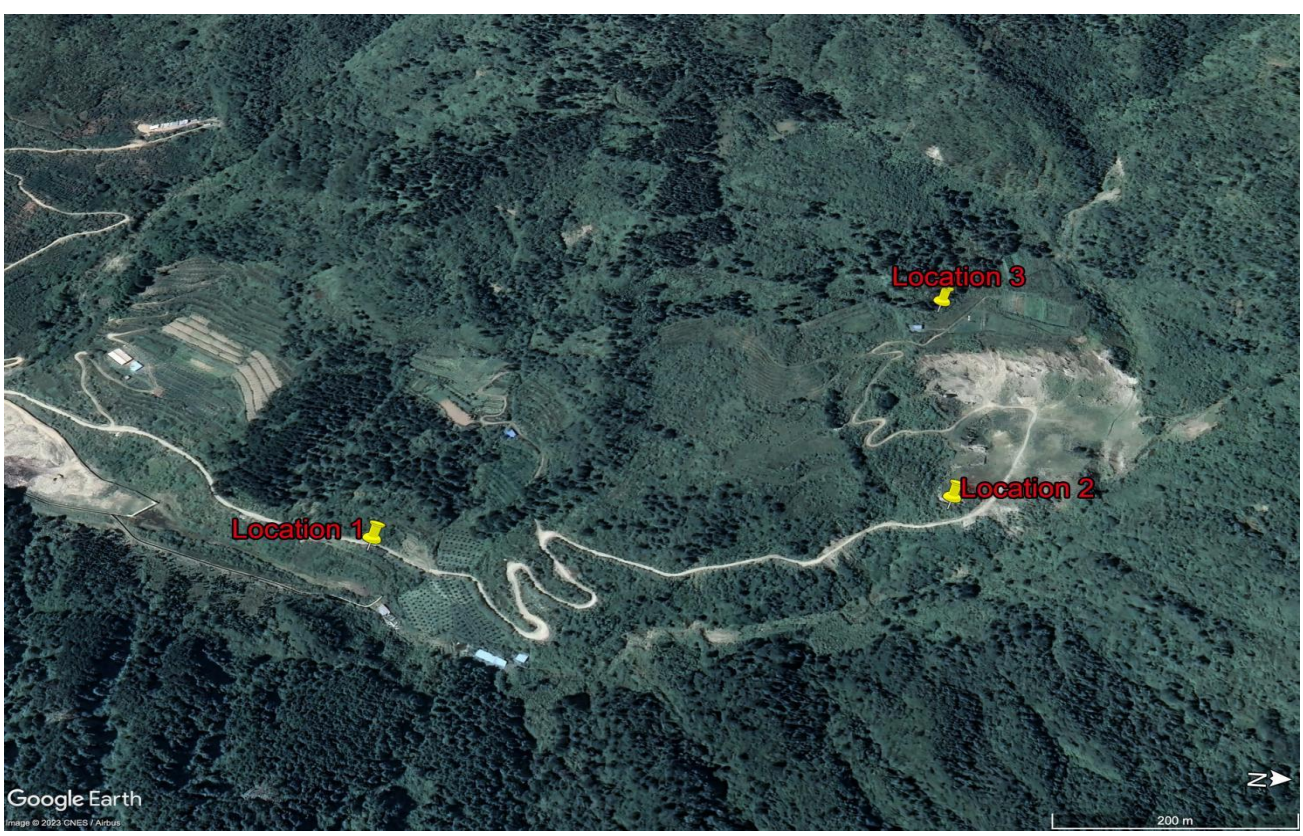

Fig. 1. The map of the three locations in Mountain Hou, Mao County, Sichuan Province.

Table 1. The environmental and anthropic properties of three *Fragaria pentaphylla* patches at three different altitudes.

Population	Location 1	Location 2	Location 3
Altitude	1901 ± 10 m	2007 ± 10 m	2087 ± 18 m
Latitude	31°42′46″N	31°43′03″N	31°43′03″N
Longitude	103°53′21E	103°53′18E	103°53′09E
Community type	Shrubbery	Meadow	Meadow
Habitat	Under the shrubs beside the ditch, with human disturbance	Near the quarry, with artificial dust and exhaust from large trucks	Sunny hillside
Patch size	280×380 cm^2	480×160 cm^2	480×200 cm^2
Extent of vegetation cover (%)	~70	~80	~90
Exposure as percent full sun (%)	70	100	100
Soil moisture	Moist	Dry	Moist
Red morph density (number per m^2)	12.78	13.15	14.69
Whie morph density (number per m^2)	2.41	9.17	1.62
Population density (number per m^2)	14.47	16.02	16.14
Habitat disturbance level	Mediate human activity was observed	High human activity was observed	Little human activity was observed

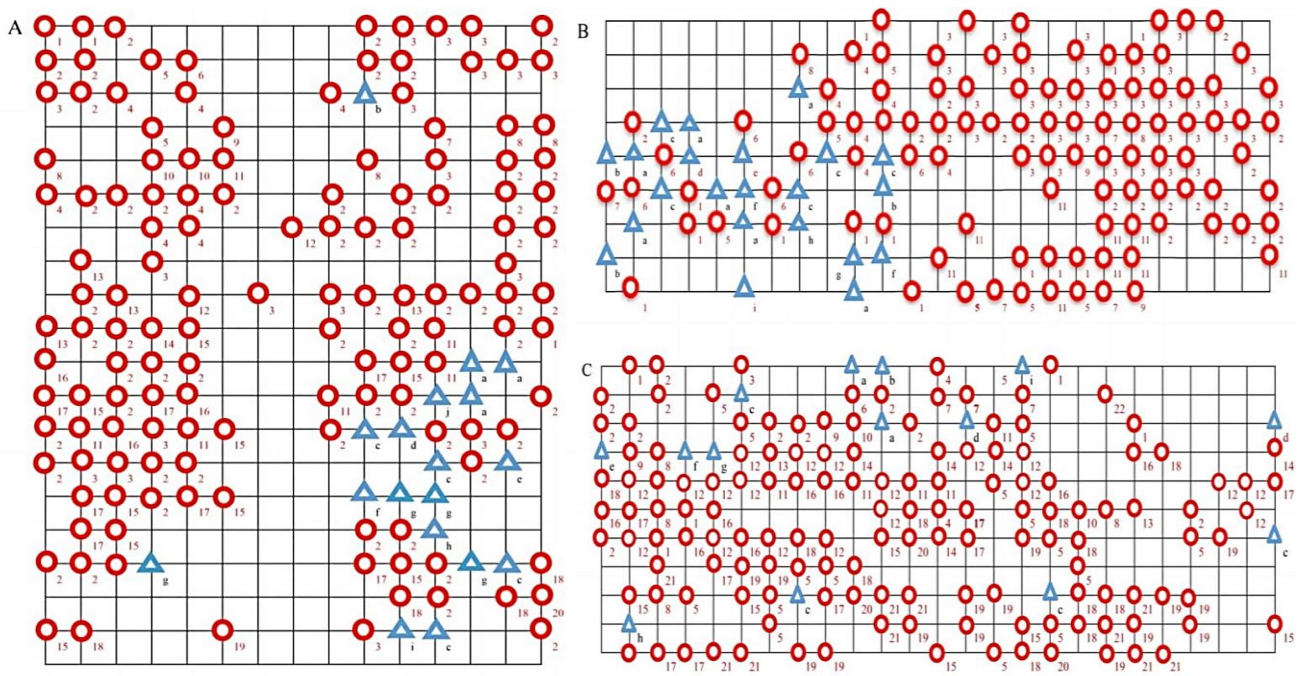

Fig. 2. The spatial distribution of ramets and genets of white and red morphs of *Fragaria pentaphylla* at location 1 (A), location 2 (B) and location 3 (C). ○=red morph of *Fragaria pentaphylla*, △=white morph of *Fragaria pentaphylla*. Different numbers (letters) indicate different genets, while the same numbers (letters) indicate the same ramets.

DNA extraction and SSR amplification: Dried leaves were ground into powder by FastPrep ® Systems (FastPrep-24, MP Biomedicals, Santa Ana, CA, USA) and DNA was extracted from 100mg of the leaf powder using a plant genomic DNA kit (Dinguo Changsheng Biotech Co., Ltd, Beijing, China). The concentration of DNA was determined using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Then DNA was diluted to a concentration of 10 $ng{\cdot}\mu L^{-1}$, and stored at a -20°C refrigerator for SSR (simple sequence repeat) amplification.

SSR primer pairs were designed according to Spigler *et al*., (2008), and primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd and labelled with 6-FAM fluorochromes. SSR amplification of *F. pentaphylla* was performed using a 20 μL polymerase chain reaction (PCR) mixture containing 2 μL 10× Taq polymerase buffer, 1.5 μL Mg^{2+} (25 mM), 0.2 μL dNTP (10 mM), 2 μL template DNA (10 $ng{\cdot}\mu L^{-1}$), 0.4 μL of each primer (10 μM) and 0.2 μL *Taq* polymerase ($5U{\cdot}\mu L^{-1}$). A negative control which consisted of ddH_2O instead of template DNA was included in the experiments and all amplifications were conducted in triplicate (Chen *et al*., 2020). The amplification reactions were performed in a T100™ Thermal Cycler (Bio-Rad, Inc., Hercules, CA, USA). The PCR conditions were 95°C (5 min), 34 cycles at 95°C (30 s), 58°C (30 s), and 72°C (45 s), with an extension at 72°C (20 min). After amplification, 1 μL product was added to 0.5 μL 500 LIZ size standard and 8.5 μL Hi-Di formamide (Applied Biosystems, Foster City, CA, USA), then the mixture was put into capillary electrophoresis of an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Finally, the band sizes were visualized and analysised by GeneMapper.

After a trial run of 30 SSR primer sets, only 5 primer sets (Table 2) which had high polymorphism, reproducible fragments and strong amplification products were selected for further analysis.

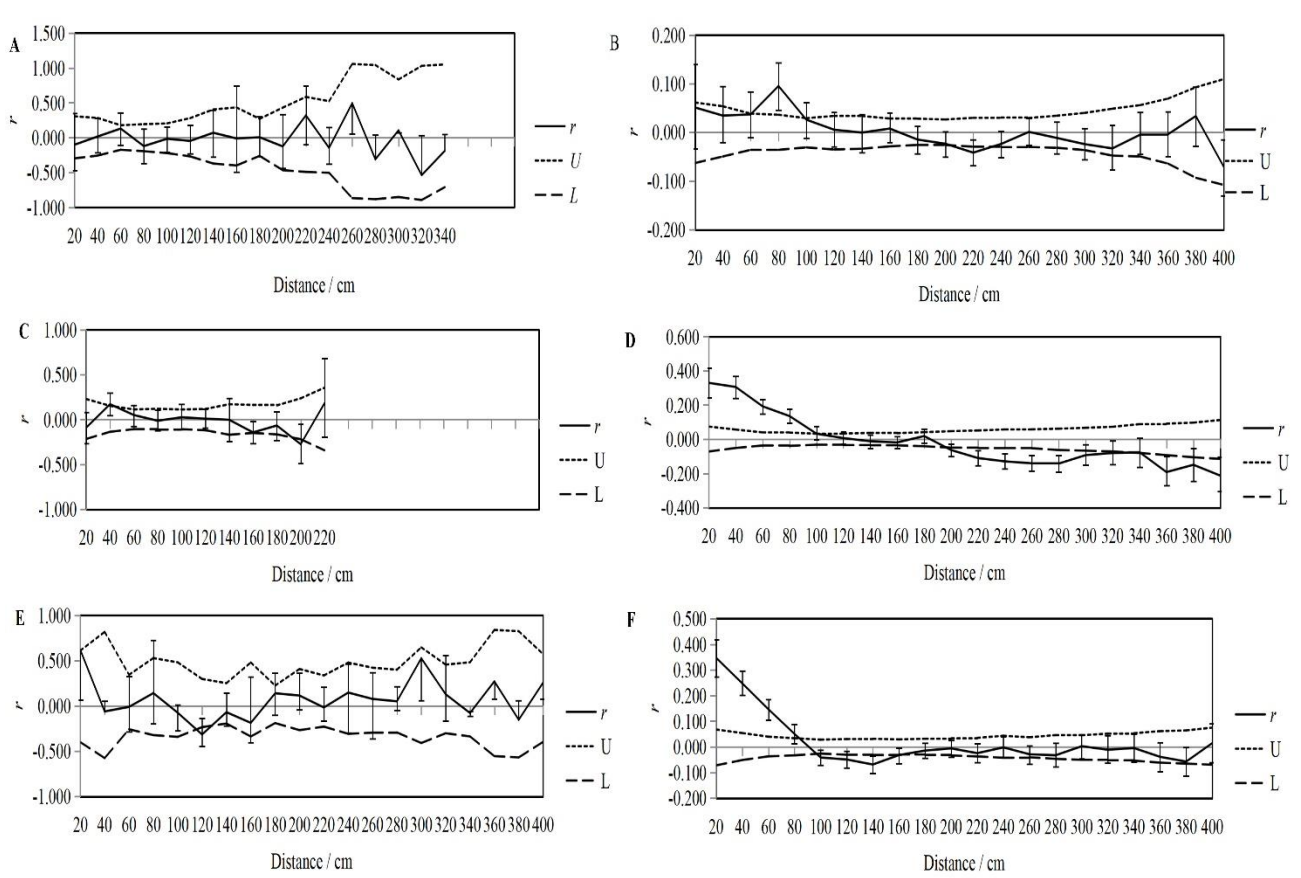

Fig. 3. Correlograms showing the spatial autocorrelation r across transects as a function of distance. U and L indicate 95% CI about the null hypothesis of a random distribution of plant, and 95% confidence error bars about *r* was determined by bootstrapping. (A) Autocorrelation for the white morph at location 1; (B) Autocorrelation for the red morph at location 1; (C) Autocorrelation for the white morph at location 2; (D) Autocorrelation for the red morph at location 2; (E) Autocorrelation for the white morph at location 3; (F) Autocorrelation for the red morph at location 3.

Table 2. Sequences of the five simple sequence repeat primer pairs used in this study.

SSR primer	Sequence 5'to 3'
Fvi 11-F	GCATCATCGTCATAATGAGTGC
Fvi 11-R	GGCTTCATCTCTGCAATTCAA
CX661264-F	GCTCTCAGATCCCTCTACCG
CX661264-R	AATTTGCAGCCATCAAGTCC
ARSFL 7-F	GCGCGCATAAGGCAACAAAG
ARSFL 7-R	GCGAATGGCAATGACATCTTCTT
CX662180-F	TTAGCCACCTTCTCCACCAC
CX662180-R	TTGGGTTGGAATTTGGAGAG
ARSFL 27-F	GCGAAGCCCAGACTCAATTACC
ARSFL 27-R	GCGTACCCGCCATTGTTAC

Data analysis: We used Microsatellite Toolkit to generate the input files for analysis. We used POPGEN32 to calculate the following parameters: $n_{a,}$ observed number of the alleles, $n_{e,}$ effective number of alleles, *I*, Shannon information index, and *H*e (expected heterozygosity) and *H*o (observed heterozygosity) in the two morphs (Borlay, 2023; Lu, 2020). GenAlEx version 6 software was used to perform AMOVA to test for genetic variation within and between populations, different morphs and different locations.

To distinguish different genets in the patchs, polymorphic loci (data from SSR) were used (Li & Dong, 2009). Individuals with the same genotypes were considered to be the same clone or genet. We used the Rclone script to evaluate clonal diversity (Arnaud-Haond & Belkhir, 2007) including these following parameters: the clonal diversity index (*R*) (Dorken & Eckert, 2001; Ellstrand & Roose, 1987), the Shannon-Wiener index estimator (*H′*) (Pielou, 1966), the Pielou evenness index(*J′*) (Pielou, 1975); the Simpson complement unbiased (*D′*) (Pielou, 1969); the Simpson complement index (*V*,) (Hurlbert, 1971; Fager 1972); and the reciprocal of Simpson index unbiased (Hurlbert, 1971; Hill, 1973).

The spatial distribution of each genet in the patch was evaluated by constructing a detailed map that identified the genotype of each ramet and enabled the distribution and spatial extent of genets to be determined (Li & Dong, 2009). The GenAlEx version 6 software was used to calculate *r* (spatial autocorrelation coefficient) for each distance class by using pairwise geographic and pairwise squared genetic distance matrices (Smouse & Peakall, 1999; Peakall & Smouse, 2006). Two methods were used for the tests of statistical significance (Peakall *et al*., 2003): random permutation and bootstrap estimates of *r*, the number of permutations and bootstraps were set to 1,000. This provides an estimate of *r* against the null hypothesis of no spatial genetic structure (*r*p). After 1,000 permutations, the *r*p's are sorted and the 25th and 975th *r*p's are used to define the upper and lower bounds of the 95%

confidence intervals, respectively. *r* was calculted for increasing distance class sizes, and *r* decreases with increasing distance class size when significant positive structure is present (Peakall *et al*., 2003).

Results

Genetic diversity of two *F. pentaphylla* morphs in populations located at different sites: Of the six genetic diversity indices, only the Na value of the red morph was significantly greater than that of the white morph (paried t-test=6.6534.103, p =0.022) (Table 3 and Table S1). There was no significantly difference in the genetic diversity of the white and red morphs between the populations at the difference locations (paired t-test, p>0.05).

AMOVA analysis showed that most variation exsited among individuals within with the red morph or white morph population (88%), while little variation exsited among red morph and white morph in the same location (5%) and among populations in different locations (7%).

Clonal diversity of two *F. pentaphylla* morphs at different altitudes: Guerrilla mode was found in both white and red morph (Fig. 2). The *J′* value of the white morph was significantly greater than that of the red morph (paried *t*-test=6.601, p =0.022), while the *R* value of the white morph was marginally significantly greater than that of the red morph (paried *t*-test=4.103, p =0.055) and the *H″* value was marginally significantly smaller (paried *t*-test=-3.478, p =0.074) (Fig. 2 and Table 4). No significant difference was found for the other clonal diversity indices (Fig. 2 and Table 4).

The *R* value of the white and red morphs located at site 2 was significantly greater than that at site 3 (paried t-test=28.256, p =0.023) (Fig. 2 and Table 4). The *J′* value of the white and red morphs located at site 1was marginally significantly smaller than those at site 2 (paried *t*-test=-8.163, p =0.078) and site 3 (paried t-test=-7.728, p =0.082), respectively (Fig. 2 and Table 4). The *D′* value of the white and red morphs located at site 1was marginally significantly smaller than that at site 3 (paried *t*-test=-11.740, p =0.054). No significant difference was found for the other clonal diversity indices (Fig. 2 and Table 4).

Fine-scale spatial genetic structure: At location 1, a significant positive correlation was found in the red morph at a distance of 60 cm, 80 cm and 100 cm with an *x*-intercept of 138.37 cm (Fig. 3A), while no significant correlation was observed in the white morph with an *x*-intercept of 70.04 cm (Fig. 3B).

At location 2, a significant positive correlation was observed in the red morph at a distance less than 100 cm with an *x*-intercept of 126.65 cm (Fig. 3C), while a significant correlation was observed in the white morph at 40 cm with an *x*-intercept of 76.12 cm (Fig. 3D).

At location 3, a significant positive correlation was observed in the red morph at a distance less than 80 cm with an *x*-intercept of 90.98 cm (Fig. 3E), while a significant correlation was observed in the white morph at 20 cm with an *x*-intercept of 38.36 cm (Fig. 3F).

The *x*-intercept of the red morph was significantly greater than that of the white morph (paired *t*-test=10.175, p=0.010), while the *x*-intercept of the two morphs at loation 1 and location 2 was marginally (paired *t*-test=5.033, p=0.125) and significantly (paired *t*-test=35.134, p=0.018) greater than that at location 3.

Table 3. The genetic diversity indices of the white and red morphs of *Fragaria pentaphylla* at the three locations.

Sites	Morph	Value	*N*	n_a	n_e	*I*	H_e	H_o	*Nei*
1	Red	Mean	136	5.4	2.4889	1.0429	0.5678	0.8588	0.5678
		SD		2.2	0.6291	0.3306	0.1522	0.2752	0.1522
1	White	Mean	18	3	2.0037	0.7656	0.4854	0.7667	0.4719
		SD		1	0.5197	0.2639	0.1463	0.3249	0.1422
2	Red	Mean	101	5.4	2.8499	1.1449	0.6218	0.8713	0.6250
		SD		2.6	0.7230	0.3274	0.1220	0.1600	0.1214
2	White	Mean	22	4	2.6094	1.0439	0.5926	0.7364	0.5791
		SD		1.4	0.9345	0.3396	0.1396	0.2988	0.1364
3	Red	Mean	141	6.2	2.4578	1.0092	0.5411	0.6326	0.5392
		SD		3.3	0.9135	0.4631	0.1920	0.3969	0.1913
3	White	Mean	14	4.2	2.9121	1.0008	0.5460	0.6714	0.5265
		SD		3.5	2.3295	0.6747	0.2283	0.3932	0.2201

N, Number of individuals; n_a, The observed number of the alleles; n_e, Effective number of alleles; *I*, Shannon information index; H_e, Expected heterozygosity; H_o, Observed heterozygosity () in the white and red morphs individually and combined

Table 4. The clonal diversity indices of the white and red morphs of *Fragaria pentaphylla* at the three locations.

Location	Morph	*N*	*G*	*R*	*H″*	*J′*	*D′*	*V*	*Hill*
1	White	18	9	0.4706	1.9269	0.8770	0.8627	0.6667	7.29
	Red	136	43	0.3111	2.8845	0.7680	0.8745	0.7621	7.97
2	White	22	20	0.9048	2.9650	0.9897	0.9913	0.5263	115.50
	Red	101	53	0.5200	3.3988	0.8561	0.9281	0.7193	13.92
3	White	14	12	0.8462	2.4410	0.9823	0.9780	0.5455	45.50
	Red	141	65	0.4571	3.7761	0.9046	0.9717	0.9309	35.38

N, The number of individuals; *G*, The number of genets; *R*, The clonal diversity index; *H″*, The Shannon-Wiener index estimator; *J′*, The Pielou evenness index; *D′*, The Simpson complement unbiased; *V*, The Simpson complement index; Hill, The reciprocal of Simpson index unbiased

Table S1. The genetic diversity indices of the white and red morphs of *Fragaria pentaphylla* at the three locations based on single SSR locus.

Location	Morph	SSR locus	*N*	n_a	n_e	*I*	H_e	H_o	*Nei*
		CX-1	136	6	2.8521	1.2215	0.6518	1.0000	0.6494
		ARSFL-7		8	3.0511	1.3254	0.6723	1.0000	0.6723
1	Red	CX-4		6	2.5257	1.0922	0.6041	0.9559	0.6041
		ARSFL-27		2	1.4287	0.4771	0.3001	0.3676	0.3001
		Fvi-11		5	2.5867	1.0984	0.6134	0.9706	0.6134
		CX-1	18	3	2.1107	0.8004	0.5413	1.0000	0.5262
		ARSFL-7		4	2.2345	0.9077	0.5683	1.0000	0.5525
1	White	CX-4		2	1.3846	0.4506	0.2857	0.3333	0.2778
		ARSFL-27		2	1.6000	0.5623	0.3857	0.5000	0.3750
		Fvi-11		4	2.6888	1.1070	0.6460	1.0000	0.6281
		CX-1	101	4	2.7868	1.1075	0.6444	1.0000	0.6412
		ARSFL-7		9	3.4816	1.4456	0.7163	1.0000	0.7128
2	Red	CX-4		6	2.7818	1.2046	0.6437	0.8812	0.6405
		ARSFL-27		2	1.7171	0.6083	0.4197	0.5941	0.4176
		Fvi-11		6	3.4822	1.3583	0.7164	0.8812	0.7128
		CX-1	22	4	2.2884	0.9379	0.5761	1.0000	0.5630
		ARSFL-7		4	2.9693	1.2073	0.6786	1.0000	0.6632
2	white	CX-4		6	4.0502	1.5025	0.7706	0.8182	0.7531
		ARSFL-27		2	1.6575	0.5860	0.4059	0.5455	0.3967
		Fvi-11		4	2.0817	0.9858	0.5317	0.3182	0.5196
		CX-1	141	3	2.0284	0.7304	0.5088	1.0000	0.5070
		ARSFL-7		11	3.6661	1.6081	0.7298	0.9574	0.7272
3	Red	CX-4		8	3.0316	1.2968	0.6701	0.7872	0.6701
		ARSFL-27		4	1.3072	0.4280	0.2350	0.2695	0.2350
		Fvi-11		5	2.2560	0.9826	0.5567	0.1489	0.5567
		CX-1	14	2	2.0000	0.6931	0.5185	1.0000	0.5000
		ARSFL-7		10	7.0000	2.1062	0.8889	1.0000	0.8571
3	White	CX-4		5	2.5455	1.1731	0.6296	0.7857	0.6071
		ARSFL-27		2	1.6000	0.5623	0.3889	0.5000	0.3750
		Fvi-11		2	1.4152	0.4692	0.3042	0.0714	0.2934

Discussion

In this study, we found that the genetic diversity was similar between the white and red morphs by showing that only the n_a value of the red morph was significantly greater than that of the white morph. We also found that the red morph had higher clonal diversity than the white morph according to the *J′* value, *R* value and *H″* values. This could be due to the characterisitcs of sexual reproduction and asexual reproduction of the two morphs. Chen *et al*., (2020) found that the offpring of the red morph produced 10% more stolons than the offspring of the white morph, suggesting that a higher investment in clonal propagation in the red morph compared to the white morph. However, there was no difference between the two morphs in terms of dry mass per seed, outcrossing rates and number of seeds, indicating that the two morphs had the similar sexual reproduction. Furthermore, as mentioned by Chen *et al*., (2020), the high clonal diversity of the red morph may also be associated with its higher abundance than the white morph (Table 1).

As we hypothesized, the significant positive correlation was found in the red morph with autocorrelation at 60-100 cm, 100 cm, and 80 cm distance, whereas in the white morph with autocorrelation at 0 cm, 40 cm, and 20 cm distance. This indicates that the red morph has stronger FSGS than the white morph. Even the lack of FSGS was detected in white morph at location 1. The *x*-intercept represents the shortest average length of genetic patches when their shape is irregular (Peakall *et al*., 2003). The *x*-intercepts of the red morph were larger than those of the white morph at three locations, indicating that the red morph would invest more in clonal growth than the white morph. The red color fruit would be preferred by the dispersers (Burns, 2015). The red morph of *F. pentaphylla* also increased the post-dispersal fitness, such as the germination rate and germination date (Chen *et al*., 2020). However, in this paper, the genetic diversity of the red morph and white morphs was not affected by any of the effects of fruit color. Chen *et al*., (2020) found that the two morphs of *F. pentaphylla* showed no difference in parameters of mating system, and neither morph exhibited biparental inbreeding based on SSR data. Thus, the stronger vegetative reproduction of the red morph may be the main reason leading to the stronger FSFS of the red morph.

In this study, we also found that no significantly difference of the genetic diversity of white and red morphs among populations at difference locations, while the clonal diversity was largest at location 2, and lowest at location 1. The habitats of *F. pentaphylla* were very different at the three locations. *F. pentaphylla* at location 1 grew with dense, heavily shaded shrubs, while *F. pentaphylla* at location 2 was distributed along the road and located near a quarry, an environment that is harsh due to the changes in the soil composition caused by artificial dust, large truck exhaust and lack of water. And *F. pentaphylla* at location

3 was distributed on the sunny slopes of a relatively open hill. Clonal propagation, especially in nutrient-poor habitats, could help plants survive in harsh environments (Klimes, 1997). A strong disturbance could increase the ability of the plants to reproduce clonally, while a weak disturbance could increase ability of the plants to reproduce secually (Shen *et al*., 2007). The harsh habitat and the strong disturbance at location 2 could cause *F. pentaphylla* to select for clonal reproduction, regardless of whether it is the red morph or the white morph, resulting in a high level of clonal diversity.

In accordance with our hypothesis, the patterns of FSGS were different among the three locations. This suggests that environmental heterogeneity has an effect on the FSGS of the white and red morphs of *F. pentaphylla*. Similar findings have been reported that environmental heterogeneity such as elevation, light, nutrient supply, and disturbance may have an impact on the FSGS of plant populations (Li & Jin, 2009; Jin *et al*., 2012). *F. pentaphylla* is a stoloniferous plant. *F. penaphylla* growing in the harsh habitat at location 2 might invest more resources in clonal growth, resulting in the strongest FSGS of the red morph at location 2. As a self-incompatible clonal plant, *F. pentaphylla* may be subject to a positive genetic density effect on fertility (Eriksson, 1993). Thus, the strongest FSGS at location 2 might be associated with the highest frequency of *F. pentaphylla* at location 2 (Table 1). In addition, we found that the *x*-intercept of the white and red morphs at loation 3 was the lowest, indicating the lowest genetic patch of *F. pentaphylla* at location 3. Field observations indicated that he light may be the main environmental factor. *F. pentaphylla* at location 3 was under a sufficient amount of sunlight, whereas *F. pentaphylla* at location 1 was under shade. The fact that *F. pentaphylla* at location 3 preferred sexual reproduction over clonal reproduction under sunny habitat could explain the lowest genetic patch at location 3.

Conclusions

Based on SSR data, we found that the genetic diversity was similar between the white and red morphs of *F. pentaphylla*, but the red morph had higher clonal diversity than the white morph. No significant difference was found in the genetic diversity of the white and red morphs among populations at difference locations, while the clonal diversity was different. The red morph had stronger FSGS than the white morph. The FSGS of *F. pentaphylla* was different among the three sites, which could be due to the different investment in vegetative growth, to the heterogeneity of the habitats. Studying FSGS can provide information for elucidating the evolutionary process of shaping populations and predicting the evolution of reproductive systems in offspring (Fuchs & Hamrick, 2010). Our results could serve as a basic reference for the study of the mechanisms underlying the adaptive strategies of the wild strawberries with different fruit colors.

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