

Dissecting the inheritance pattern of anemone type and the relevant floral traits of chrysanthemum in multiple segregating F1 populations

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Abstract

Anemone type, featuring prominent and colored in tubular florets, is an attractive flower shape in chrysanthemums and thus desired by breeders and consumers. Understanding the genetic basis of anemone-type flowers in chrysanthemum is crucial for breeding success. The current study conducted nine cross combinations from seven parents to investigate segregation patterns, parental effect, and the relationships between the heterosis of six tubular floral traits and parental genetic distance. The results showed significant ($P < 0.05$) differences between the parents and crosses in various tubular floral traits, and transgressive segregation was observed in both directions for most traits. The considerable variation was revealed for almost floral traits, thus indicating the substantial potential for selecting lines with the desirable traits. The segregation ratio of anemone to nonanemone followed 3:1 in anemone × anemone crosses and 1:3 in either nonanemone × anemone or anemone × nonanemone crosses, except for two reciprocal crosses derived from the parents 'Nannong Xuefeng' and 'QX096'. The F_1 hybrids' performance was more inclined towards a vulnerable parent and involved less influence of maternal or paternal effects, displaying some extended declines and negative mid-parent heterosis (MPH). The phenotypic genetic distance was significantly negatively correlated with MPH for floral traits except for style length and disk flower diameter. In contrast, we observed no significant correlation between molecular marker-based genetic distance and MPH for all floral characteristics. The findings of the current work provide insights into the complex inheritance pattern of flower shapes and help achieve desirable improvement in anemone-type chrysanthemums.

Introduction

Chrysanthemum (*Chrysanthemum grandiflorum* Ramat.) has become a highly prized ornamental crop in China and worldwide due to plenty of colors, diversified flower shapes, and excellent decorative value (Zhang et al. 2010; Chong et al. 2019). Conventional intercultivar hybridization is an essential strategy for breeding chrysanthemum new varieties. In breeding practices, parental selection and understanding of inheritance mode are prerequisites for achieving satisfactory characteristics of interest. Anemone type, characterized by elongated and colored tubular florets in a prominent flower center, is one of the most popular flower shapes in the fresh-cut flower market (Anderson 2006; Chen et al. 2009; Li et al. 2010). However, lacking essential information on the inheritance pattern of anemone-related floral traits has impeded the breeding efficiency of the chrysanthemum breeding program.

The development of cultivars with novel flower forms is of practical significance for breeding improvement (Song et al. 2018; Wang et al. 2020; Li et al. 2021). Mastering the inheritance behavior between crossing parental cultivars can drastically accelerate the selection of desired traits in offspring. Simple genetic control has been reported for flower forms, including carnation (Nimura et al. 2008; Yagi et al. 2014), periwinkle (Kulkarni et al. 2005), *Matthiola incana* (Nakatsuka and Koishi 2018), safflower (Golkar et al. 2010) and sunflower (Cvejiä et al. 2016). A few studies have addressed the inheritance of flower shapes in chrysanthemums. According to Lim et al. (2014) and Song et al. (2018), double to single flowers were not incomplete dominance in segregating populations. Additionally, Lim et al. (2014) also suggested that the shape of the ray floret tips seemed to be controlled by a single gene, while the level of twisting of the ray floret showed a significant deviation from the expected 3:1 or 1:1 ratio. Lately, Yang et al. (2019a) indicated that the segregation ratio of anemone to nonanemone type was fit with 1:1 of the anemone type 'Nannong Xuefeng' × the nonanemone type 'QX096'; however, the ratio of the cross between parents with anemone types slight deviated from Mendel's segregation manner 3:1 by Chi-square test.

Maternal inheritance is a common phenomenon in nature, representing the external phenotypic traits of offspring mainly transmitted from their maternal parent (Badyaev 2013). Plant maternal effects profoundly influence natural

selection, biological evolution, and offspring's gene action (Wu et al. 2012; Ferfua and Vannozi 2015). Understanding the genetic mechanism of maternal effects will help formulate more effective breeding strategies and increase genetic gain. Previous studies reported maternal effects on sugar content in sugarbeet root (Jassem et al. (2000), fatty acid composition in soybean (Gilsinger et al. 2010), and organic acid content in pear (Liu et al. 2016). Only a few studies have investigated maternal effects on floral traits in chrysanthemum. Chen et al. (2003) found that the red color had a strong hereditary ability and showed significant maternal inheritance in F_1 progenies. However, whether maternal parents affect the inheritance of anemone flower type is rarely known.

Heterosis, a biological phenomenon of higher vigor in F_1 plants than in their parental lines, can be attributed to non-additive and additive genetic effects (Yao et al. 2015). Utilizing the heterosis has become a priority for increasing the productivity of different crops, e.g., maize (Giraud et al. 2017), rapeseed (Tian et al. 2017), and wheat (Jiang et al. 2017). In chrysanthemum, heterosis has been widely observed for horticultural and resistance traits (Zhang et al. 2011; Yang et al. 2015; Su et al. 2016; Tang et al. 2015). Accurately predicting and thus selecting excellent hybrids of interest will enhance the efficiency of breeding activities. Morphological evaluation of hybrid performance has been widely undertaken, whereas it needs considerable time and effort and is liable to be affected by the environment. Genetic distance (GD), estimated by molecular markers, proved effective in parental selection and heterosis prediction. Recently, Su et al. (2017) successfully predicted heterosis of waterlogging tolerance traits by estimating pairwise parental genetic distance resulting from QTL-linked markers.

The objectives of this study were 1) to investigate genetic variation of anemone type flower in multiple crosses with different backgrounds, 2) to better understanding of whether a parental effect is involved in anemone flower shape and its attributing tubular floral traits, and 3) to determine the correlation between heterosis and parent-wise genetic distance for the sake of prediction of heterosis in future breeding practices. Results from the present study cast light on the inheritance pattern of anemone flower shape and its relevant tubular floral traits and functions in developing new anemone-type chrysanthemum cultivars.

Materials And Methods

Parental cultivars and hybridization

The quantitative traits description of the parental materials of chrysanthemum cultivars, 'QX021', 'QX081', 'QX096', 'QX097', 'QX098', 'Nannong Xuefeng', and 'Monalisa' were given in Table 1. 'Nannong Xuefeng' (renamed 'XF') and 'Monalisa' (renamed 'MB') are typical anemone type, and the remaining five cultivars the nonanemone type (Fig. 1). Nine cross combinations were subsequently obtained via artificial hybridization, including 'QX021' × 'MB' (Co1), 'QX081' × 'MB' (Co2), 'QX097' × 'MB' (Co3), 'XF' × 'MB' (Co4), 'XF' × 'QX096' (Co5), 'XF' × 'QX098' (Co6), and three reciprocal crosses, 'MB' × 'QX097' (Bco3), 'MB' × 'XF' (Bco4) and 'QX096' × 'XF' (Bco5). The former six F_1 populations (Co1-6) were recently produced by Su et al. (2017) to dissect the inheritance of waterlogging tolerance (Su et al. 2017, 2018), of which Co4 and Co5 were used to develop an anemone-specific SCAR marker (Yang et al. 2019a). The parental cultivars and their F_1 progenies are maintained at Nanjing Agricultural University's Chrysanthemum Germplasm Resource Preserving Centre (Nanjing, China). The accessions were raised in a randomized complete block with three replicates. Six plants represented each replicate of each entry. The inter-plant and inter-row spacing were set to 30 cm and 20 cm, respectively. The field management followed standard commercial practices.

Table 1
The major floral traits of the seven parental cultivars of cut chrysanthemum

Parent	Flower shape	LLL ^a (mm)	TFL (mm)	TFW (mm)	SL (mm)	TFL/SL	DFD (mm)
QX021	nonanemone	0.79 ± 0.02d ^b	3.74 ± 0.08f	1.46 ± 0.04d	5.11 ± 0.10d	0.73 ± 0.02d	11.44 ± 1.01f
QX081	nonanemone	0.97 ± 0.02c	5.04 ± 0.17d	1.65 ± 0.03d	6.36 ± 0.33c	0.80 ± 0.04c	14.35 ± 0.98e
QX096	nonanemone	1.07 ± 0.04bc	6.08 ± 0.32c	1.96 ± 0.03c	7.51 ± 0.19a	0.81 ± 0.04c	21.48 ± 0.89c
QX097	nonanemone	0.95 ± 0.02c	4.30 ± 0.97e	1.90 ± 0.05c	6.79 ± 0.10b	0.63 ± 0.18e	21.59 ± 1.43c
QX098	nonanemone	0.90 ± 0.06c	3.78 ± 0.97f	1.45 ± 0.03d	5.54 ± 0.26c	0.68 ± 0.18e	18.85 ± 1.02d
Nannong Xufeng	anemone	1.96 ± 0.21b	11.44 ± 0.58b	2.22 ± 0.03b	5.74 ± 0.26c	2.09 ± 0.14b	27.90 ± 1.32b
Monalisa	anemone	3.08 ± 0.14a	15.60 ± 0.57a	3.96 ± 0.30a	5.88 ± 0.25c	2.65 ± 0.07a	35.10 ± 1.02a
^a LLL, Longest lobe length; TFL, Tubular floret length; TFW, Tubular floret width; SL, Style length; TFL/SL, Tubular floret length/Style length; DFD, Disk flower diameter							
^b The data with different lower case letters in same column indicates the significant difference at 0.05							

Floral Traits Investigation

At the flowering stage in 2017 and 2018, we scored the tubular floral traits relevant to anemone type as previously reported by Yang et al. (2019a). The tubular floral traits included the longest lobe length (LLL, mm), tubular florets length (TFL, mm), tubular florets width (TFW, mm), style length (SL, mm), tubular floret length/style length (TFL/SL) and disc flower diameter (DFD, mm). For DFD, three randomly selected inflorescences were measured for each entry. The other floral traits were tested five times for an inflorescence, thus resulting in 15 measurements per trait for the three inflorescences. As illustrated in our previous researches (Chen et al. 2009; Yang et al. 2019a), we categorized the flowers as anemone type if TFL/SL > 1, and otherwise the non-anemone type below the threshold.

Statistical analysis

The descriptive phenotypic data were statistically analyzed using routines implemented in Microsoft Excel 2012, SPSS v20.0 software (SPSS, Chicago, USA), and the R package (R Core Team 2013). Additionally, the hybrids' heterosis was estimated by mid parent heterosis (MPH) and calculated as follows: MPH (%) = $(F_1 - MP)/MP \times 100$, where F_1 is the average performance of offspring for each combination, $MP = (P_1 + P_2)/2$, in which P_1 and P_2 are the morphological values of both parental lines. The parent-wise genetic distance was estimated from six tubular floral traits and SNPs. The Euclidean distance based on morphological data was obtained after standardized transformation using SPSS 20.0 software. A set of 38,655 SNPs screened by Chong et al. (2017, 2019) was employed to measure Nei's genetic distance (Nei 1972). The clustering using the unweighted pair group method analysis (UPGMA) was performed in the NTSYS-pc 2.2 software package (Rohlf 2000). Mantel test (Mantel 1967)

was employed to reveal correlations between the morphological and genotypic data sets. The correlation between heterosis and parent-wise genetic distance was examined through the Pearson's test in SPSS software.

Results

Phenotypic performance

The phenotypic values of six tubular floral traits of the seven parents and their F₁ progenies were summarized in Table 1 and Table 2. The result revealed highly significant differences among the parents. Two anemone-type parents, 'XF' and 'MB', showed better performances, and their mean values were significantly ($P < 0.05$) higher than the other nonanemone parental cultivars for all floral traits except SL. On the contrary, 'QX021' had the weakest phenotypic expression in all floral traits. For the F₁ progenies, a significant morphological difference was observed for all examined traits. Progenies from Co4 always had excellent expression in the other five attributes other than SL, whereas Co1 and Co6 yielded the lowest phenotypic performance. In general, there was a wide genetic variation in each trait, and the average coefficient of variation (CV) fluctuated from 8.7% (SL) to 41.6% (LLL) across the crosses. The average CV of all hybrid populations reached ~ 30%, implying a remarkable potential for desirable trait improvement via crossbreeding. The offspring from different parents displayed various CVs given the same floral characteristics. For example, Co5 exhibited the most extensive CV of 45.2%, whereas Co6 showed the lowest CV of 11.6% for TFL/SL. This suggests that parent selection in crossbreeding is relevant to the breeding success of anemone-type chrysanthemums.

Table 2

Phenotypic statistics for the major floral traits in F₁ segregating population of different cross combinations

Trait	Combination	Range	Mean	SD	CV (%)	MP	MPH (%)	< Low parent value (%)	Between parent value (%)	> High parent value (%)
LLL ^a (mm)	Co1	0.59 ~ 3.40	1.12 ± 0.06bc ^b	0.51	45.54	1.93	-59.36	23.61	75	1.39
	Co2	0.60 ~ 2.13	1.12 ± 0.04bc	0.35	31.25	2.02	-52.29	37.35	62.65	0
	Co3	0.83 ~ 4.09	1.34 ± 0.07b	0.63	47.01	2.01	-52.89	18.52	77.78	3.7
	Co4	0.62 ~ 6.77	2.34 ± 0.11a	1.37	58.55	2.52	-22.03	51.01	23.49	25.5
	Co5	0.50 ~ 3.61	1.40 ± 0.07b	0.65	46.52	1.51	-7.67	43.01	38.71	18.28
	Co6	0.49 ~ 2.03	0.89 ± 0.04c	0.13	14.99	1.43	-37.99	72.86	25.71	1.43
TFL (mm)	Co1	3.69 ~ 10.04	5.13 ± 0.16d	1.39	27.1	9.67	-61.36	1.39	98.61	0
	Co2	4.49 ~ 14.00	6.18 ± 0.24c	2.2	35.6	10.32	-51.12	33.73	66.27	0
	Co3	4.53 ~ 18.42	7.09 ± 0.35c	3.15	44.43	9.95	-56.76	0	97.53	2.47
	Co4	4.14 ~ 22.69	11.25 ± 0.35a	4.25	37.78	13.52	-15.39	46.98	36.91	16.11
	Co5	4.15 ~ 20.65	8.44 ± 0.39b	3.72	44.13	8.76	-3.64	36.56	41.94	21.51
	Co6	3.10 ~ 10.61	4.87 ± 0.20d	2.94	60.38	7.61	-36.04	20	80	0
TFW (mm)	Co1	1.38 ~ 2.81	1.83 ± 0.03d	0.3	16.39	2.71	-46.29	9.72	90.28	0
	Co2	1.46 ~ 3.30	1.93 ± 0.04cd	0.32	16.58	2.81	-41.08	8.43	91.57	0
	Co3	1.36 ~ 4.54	2.14 ± 0.06b	0.56	26.17	2.93	-35.17	34.57	62.96	2.47

^a Longest lobe length (LLL), Tubular floret length (TFL), Tubular floret width (TFW), Style length (SL), Tubular floret length/Style length (TFL/SL) and Disk flower diameter (DFD)

^b The data with different lower case letters in same column indicates the significant difference at 0.05

Trait	Combination	Range	Mean	SD	CV (%)	MP	MPH (%)	< Low parent value (%)	Between parent value (%)	> High parent value (%)
	Co4	1.09 ~ 4.00	2.58 ± 0.05a	0.65	25.19	3.09	-28.28	28.19	71.14	0.67
	Co5	1.04 ~ 4.21	2.06 ± 0.07bc	0.67	32.67	2.09	-1.15	52.69	11.83	35.48
	Co6	0.97 ~ 2.59	1.51 ± 0.05e	0.16	10.38	1.83	-17.53	62.86	30	7.14
SL (mm)	Co1	4.58 ~ 8.35	5.95 ± 0.07c	0.62	10.42	5.5	-7.07	5.56	48.61	45.83
	Co2	4.07 ~ 7.64	6.40 ± 0.05b	0.49	7.66	6.12	3.82	7.23	39.76	53.01
	Co3	5.69 ~ 8.34	6.88 ± 0.05a	0.48	6.98	6.34	7.11	2.47	39.51	58.02
	Co4	3.54 ~ 7.21	5.82 ± 0.06c	0.71	12.2	5.81	-1.3	42.28	10.74	46.98
	Co5	4.03 ~ 8.66	6.77 ± 0.06a	0.62	9.22	6.62	2.21	3.23	91.4	5.38
	Co6	3.59 ~ 6.91	5.14 ± 0.07d	0.31	6.01	5.64	-8.86	77.14	10	12.86
TFL/SL	Co1	0.63 ~ 1.73	0.87 ± 0.03c	0.25	28.74	1.69	-56.64	31.94	68.06	0
	Co2	0.69 ~ 2.55	0.98 ± 0.04c	0.39	39.8	1.72	-53.78	39.76	60.24	0
	Co3	0.69 ~ 2.66	1.04 ± 0.05c	0.47	45.19	1.64	-61.76	0	98.77	1.23
	Co4	0.67 ~ 4.13	2.01 ± 0.07a	0.88	43.78	2.32	-14.12	49.66	25.5	24.83
	Co5	0.71 ~ 3.46	1.25 ± 0.06b	0.58	46.4	1.4	-10.56	16.13	69.89	13.98
	Co6	0.68 ~ 2.04	0.95 ± 0.04c	0.11	11.56	1.34	-28.96	0	98.57	1.43
DFD (mm)	Co1	10.00 ~ 22.52	14.97 ± 0.36d	3.04	20.31	23.56	-51.43	12.5	87.5	0
	Co2	11.72 ~ 33.24	17.32 ± 0.41c	3.71	21.42	25.01	-42.64	13.25	86.75	0

^a Longest lobe length (LLL), Tubular floret length (TFL), Tubular floret width (TFW), Style length (SL), Tubular floret length/Style length (TFL/SL) and Disk flower diameter (DFD)

^b The data with different lower case letters in same column indicates the significant difference at 0.05

Trait	Combination	Range	Mean	SD	CV (%)	MP	MPH (%)	< Low parent value (%)	Between parent value (%)	> High parent value (%)
	Co3	12.23 ~ 43.63	21.30 ± 0.69b	6.17	28.95	28.63	-24.6	69.14	27.16	3.7
	Co4	12.72 ~ 42.56	24.61 ± 0.57a	6.97	28.33	31.79	-12.25	69.13	24.83	6.04
	Co5	10.30 ~ 49.15	22.56 ± 0.73b	7.02	31.1	24.69	-8.62	55.91	23.66	20.43
	Co6	10.27 ~ 21.26	14.16 ± 0.34d	8.06	56.9	23.37	-39.43	91.43	8.57	0
^a Longest lobe length (LLL), Tubular floret length (TFL), Tubular floret width (TFW), Style length (SL), Tubular floret length/Style length (TFL/SL) and Disk flower diameter (DFD)										
^b The data with different lower case letters in same column indicates the significant difference at 0.05										

Heterosis

The heterosis estimation of six tubular floret-related traits was shown in Table 2. The MPH values for most attributes were negative, except Co2, Co3, and Co5 for SL, indicating the depression phenomenon in segregating F₁ populations. Additionally, the MPH values of different crosses for each trait varied in magnitude. Compared with other F₁ populations, Co5 always had relatively high scores, whereas Co1 displayed a significant recession trend for most floral traits. According to our statistical results, most progenies were intermediate between their parents in the examined traits, and transgressive segregation in both directions was observed. More significantly, super-parental lines existed in all combinations for SL, and their proportion of the over-high-parent individuals ranged from 5.38% (Co5) to 58.02% (Co3). However, there were few over-high-parent individuals in Co1 and Co2 for the investigated floral traits except SL.

Flower Shape Heritage

The flower segregations of anemone and nonanemone types were presented in Table 3 and Fig. 2. In nonanemone × anemone or anemone × nonanemone crosses, the majority (over ~ 75%) of the progenies displayed nonanemone type. The anemone and nonanemone types in those crosses were segregated in a 1:3 ratio according to the χ^2 test result. However, Co5 and Bco5 from parents 'XF' and 'QX096' transmitted more anemone types in their segregating populations, and the segregation ratio of anemone to nonanemone types was 1:1 and 3:1, respectively. In addition, crossing parents sharing the same anemone type also yielded progenies with nonanemone shapes. For example, the anemone × anemone crosses, Co4 and Bco4, were segregated into the anemone and nonanemone types, and here the anemone dominated in the progenies, both fitting a ratio of 3:1.

Table 3
The segregation of flower type in F₁ population derived from different cross combinations

Cross combinations	Code	Number	Anemone	Nonanemone	Ratio	χ^2
QX021 × Monalisa	Co1	72	14	58	1:3	1.18 (3.84) ^a
QX081 × Monalisa	Co2	83	17	66	1:3	0.90 (3.84)
QX097 × Monalisa	Co3	81	17	64	1:3	0.70 (3.84)
Nannong Xuefeng × Monalisa	Co4	149	122	27	3:1	3.76 (3.84)
Nannong Xuefeng × QX096	Co5	93	52	41	1:1	1.30 (3.84)
Nannong Xuefeng × QX098	Co6	70	22	48	1:3	1.54 (3.84)
Monalisa × QX097	Bco3	87	24	63	1:3	0.31 (3.84)
Monalisa × Nannong Xuefeng	BCo4	91	70	21	3:1	0.18 (3.84)
QX096 × Nannong Xuefeng	BCo5	87	64	23	3:1	0.10 (3.84)

^a When the degree of freedom equals to 1, the threshold is 3.84 at 0.05 level

Parental Effect

To analyze the parental effect, we analyzed the distribution patterns of six floral traits among crosses involving different parent types (Fig. 3). Among the crosses (Co1-4) with the same male parent but different female parents, the F₁ progenies were mainly concentrated between the two parents and much closer to females for the floral traits other than SL, implying that female parent played a dominant role in hybrids' performance. On the other hand, among the crosses (Co4-6) with the same female parent but different male parents, the F₁ progenies displayed variable distributions. Co4 individuals tended to their female parent, whereas the performance of Co5 and Co6 were much closer to the males.

To further confirm the presence of parental effect in the inheritance of anemone flower shape, we compared three pairs of reciprocal crosses, Co3 vs. Bco3, Co4 vs. Bco4, and Co5 vs. Bco5. Co3 and Bco3 derived from contrasting flower shape parents exhibited a similar segregation ratio for anemone and nonanemone types but different for the reciprocal crosses of the same type Co5 and Bco5. In addition, Co4 and Bco4 derived from both anemone type parents segregated similarly with anemone types occupying the majority of the progenies. These findings were confirmed by the frequency distribution of the anemone type-attributing tubular floral traits in the reciprocal crosses (Fig. 4). For example, in TFL/SL, Bco5 lay the most significant proportion in the range over 2.50 (~ 25%), a considerable increase compared to the Co5. Comparatively, the highest proportion of Co5 was primarily located in the range 1.0 ~ 1.2 (~ 23%). Overall, most traits of the F₁ progenies displayed similar phenotypic values and segregation in reciprocal crosses except Co5 vs. Bco5, implying the equal parental contribution to F₁ performance (Fig. S1). Nevertheless, the anemone-type parent 'XF' is dominant to the nonanemone type in their progenies of Co5 vs. Bco5. Combining the above results, it seems that hybrids' performance was more inclined towards a vulnerable parent and involved less influence of maternal effect.

Correlation Between Parent-wise Genetic Distance And Heterosis

The phenotype- and SNP- based parent-wise genetic distances, termed PD, GD, respectively, were listed in Table 4. The PD ranged from 0.00 to 1.00 and averaged at 0.44. The GD ranged from 0.35 to 0.45, with a mean of 0.43. Both indicated a moderate level of polymorphism among parents. However, the goodness-of-fit between PD and GD matrices revealed a very low correlation ($r = 0.0330$, $P > 0.05$). As a result, the cluster analyses based on PD and GD provided somewhat different divisions of the parents (Fig. S2). The PD dendrogram could separate anemone or nonanemone type parents, whereas the GD dendrogram did not follow the PD-based clustering.

Table 4

Person correlation coefficients among the phenotypic value, heterosis and parental genetic distance (PD and GD) for six anemone-related traits

Trait	LLL ^a	TFL	TFW	SL	TFL/SL	DFD
PD						
Mean	-0.48	-0.68	-0.26	0.35	-0.67	-0.45
MPH	-0.89**	-0.91**	-0.77*	0.22	-0.95**	-0.74
GD						
Mean	-0.35	-0.57	-0.49	-0.33	-0.33	-0.42
MPH	0.24	0.19	0.36	-0.69	0.25	-0.11
^a Longest lobe length (LLL), Tubular floret length (TFL), Tubular floret width (TFW), Style length (SL), Tubular floret length/Style length (TFL/SL) and Disk flower diameter (DFD)						
*, ** Significance at $P < 0.05$ and $P < 0.01$, respectively						

The correlation of parent-wise genetic distances with hybrid performances and heterosis was listed in Table 4. PD was significantly negative correlated with MPH for LLL, TFL, TFW and TFL/SL ($-0.95 < r < -0.77$, $P < 0.05$). A negligible correlation was revealed between PD and mean values, whereas no significant correlation was observed between GD and MPH and between GD and mean values of all traits.

Discussion

Elucidating the genetic control of heritable traits of plants is vital for improvement. The inheritance of flower shapes has been investigated in various ornamental plant species, e.g., the recessive allele was responsible for the single flower phenotype in carnation (Yagi et al. 2014) and *Petunia hybrida* (Liu et al. 2016). In contrast, the double-flower phenotype is a single recessive trait in gentians (Tasaki et al. 2017) and peach (Meng et al. 2019). For chrysanthemums, research on the inheritance mode of flower shape has progressed rather slowly, probably attributing to its nature of genome complexity and high heterozygosity (Anderson and Ascher 2000; Tang et al. 2009; Zhang et al. 2011; Su et al. 2016). Furthermore, several papers have attempted to uncover the genetic law. Dejong and Drennan (1984) reported that singleness was partially dominant to doubleness. Similarly, Lim et al. (2014) suggested an incomplete dominance for double and single flowers in the 'Falcao' × 'Frill Green' population. Zhu et al. (2012) reported that the hybrids differentiated obviously from their parents and represented three flower types, including standard anemone type, mid-anemone type, and non-anemone type, and most were anemone type in a backcross population. The current work found that the anemone flower shapes in hybrids were not entirely

segregated, accompanied by the generation of intermediate types. In most crosses of contrasting flower type parents, anemone and nonanemone types were segregated into a 1:3 ratio, suggesting the dominance of nonanemone type over anemone type. Comparatively, the anemone and nonanemone segregation ratio in the two crosses Co5 and Bco5, derived from parents 'XF' and 'QX096' also with contrasting flower types, deviated from the above separation ratio, but following 1:1 and 3:1 ratios, respectively. It is noteworthy that the anemone × anemone crosses also generated nonanemone-type flowers, with an anemone to nonanemone segregation ratio of 3:1. Therefore, the current research suggested that the inheritance of anemone flower shape is far more complex than expected and might be associated with more than one gene or be modified by different genes. The findings may reflect, to some extent, the complex genetic effects identified for the tubular floral traits relevant to anemone type in our recent research (Yang et al. 2019b).

Maternal inheritance is the non-Mendelian transmission of traits from mothers to their offspring (Lande and Kirkpatrick 1990). It has important implications for evolution in natural populations and practical applications in the economic improvement of species (Chandnani et al. 2017). In chrysanthemum, maternal inheritance has been proposed for flat-and spoon-type ray floret (Xu et al. 2000) and red color (Chen et al. 2003). In the current research, an important goal was to dissect the effect of maternal inheritance on anemone-type chrysanthemum. From the crosses derived from the same male anemone type parent and different female parents (Co1, Co2, Co3, and Co4) or from the same female anemone type parent and the different male parent (Co4, Co5, and Co6), we could observe a maternal or paternal effect on the inheritance of anemone type flower. To further insight into the parent effect, we produced three sets of reciprocal crosses (Co3 vs. Bco3, Co4 vs. Bco4, and Co5 vs. Bco5) with contrasting. In the light of the similar segregation in the reciprocal crosses, say Co3 vs. Bco3 and Co4 vs. Bco4 derived from contrasting parents, however, it seems difficult to decide on the maternal or paternal inheritance mode for anemone flower shape. Therefore, we cannot conclude the inheritance mode of anemone-type flower due to the segregation complexities in the current work. To our expectation, crosses Co5 vs. Bco5 derived from both anemone type parents generated more anemone type progenies, casting light on the future breeding success of anemone-type chrysanthemums. In other words, it would be fruitful to develop desirable anemone-type varieties by crossing anemone-type parents.

The hybrids' performance displayed extensive transgressive segregation and significant heterosis in chrysanthemum (Zhang et al. 2011; Yang et al. 2015; Su et al. 2017). However, most floral traits in the current crosses showed some extended decline and exhibited a negative MPH value. This contradiction may be due to the differences in species, materials, morphological characteristics, or environmental factors involved in the above studies. Moreover, high CV values were observed in the F₁ populations, indicating significant potential for selecting elite hybrids with desirable flower types. Estimating the genetic distance between parents is entirely meaningful for predicting heterosis. The morphological traits-derived genetic distance is widely applied for the advantage of being convenient and straightforward (Espósito et al. 2014). The phenotypic parent-wise genetic distance (PD) was significantly and negatively correlated with MPH for LLL, TFL, TFW, and TFL/SL in our study. However, PD and mean values of hybrid progenies were not significantly correlated for all investigated floral traits (Table 4). These findings are in accordance with Riday et al. (2003) and Geleta et al. (2004), who found that PD showed a significant correlation with tested agronomic traits in *Medicago stiva* and pepper. Nevertheless, Wegary et al. (2013) observed that non-significant correlation between PD and heterosis in maize. Hence, our results suggested the possibility of predicting the heterosis of floral traits by PD.

Previous research has proposed that, molecular markers could improve the heterosis prediction efficiency (Buti et al. 2013; Frisch et al. 2010; Sang et al. 2015). Huang et al. (2015) and Tian et al. (2017) proposed that selecting

favoring loci from molecular makers would effectively facilitate the prediction of heterosis. In chrysanthemum, Su et al. (2016) found that the genetic distance estimated by the QTL-linked markers could better predict heterosis of waterlogging tolerance traits. In our case, the SNPs-based parent-wise genetic distance did not significantly correlate with heterosis in the current study. Thus, it did not satisfactorily predict heterosis and average phenotypic values concerning flower type traits. Weak correlations were also reported in crops including maize (Devi and Singh 2010; Ndhlela et al. 2015), rapeseed (*Brassica napus* L.) (Tian et al. 2017) and wheat (Chen et al. 2010), and pepper (Geleta et al. 2004). The low level of correlation could result from several reasons, such as a lack of corresponding genetic marker associated with genes controlling the target traits and unequal genome coverage, which would contribute to F₁ performance and heterosis. Otherwise, it may also result from an extensive genetic differentiation among inter-specific hybridization progenies and genetic × environmental interactions (Ndhlela et al. 2015). In future, implementing DNA markers associated with genes responsible for the characteristics of interest, combined with the combining ability analysis, may be a promising approach for heterosis prediction in chrysanthemum.

In conclusion, the present study represented a worthwhile attempt to assess the inheritance pattern of anemone flower shape, its related tubular floral traits, and the prediction of heterosis. The majority of the assayed floral traits exhibited considerable variation, underlying the potential for future improvement of desirable chrysanthemums. The hybrids' performance involved less influence of maternal or paternal effects but related to a specific parental cultivar. The morphology other than the genome-wide SNP-based parent-wise genetic distance is promising in predicting the heterosis. The outcome of this study help understand the inheritance of flower type in chrysanthemums and lays a foundation for parental selection and genetic improvements of desired traits.

Author contribution statement FZ conceived and designed the project, FC, WF, and ZG provided the materials, XY, JS, and YQ conducted experiments, XY and FZ analyzed the data and wrote the manuscript. All authors read and agreed to the submitted version of the manuscript.

Declarations

Author contribution statement FZ conceived and designed the project, FC, WF, and ZG provided the materials, XY, JS, and YQ conducted experiments, XY and FZ analyzed the data and wrote the manuscript. All authors read and agreed to the submitted version of the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

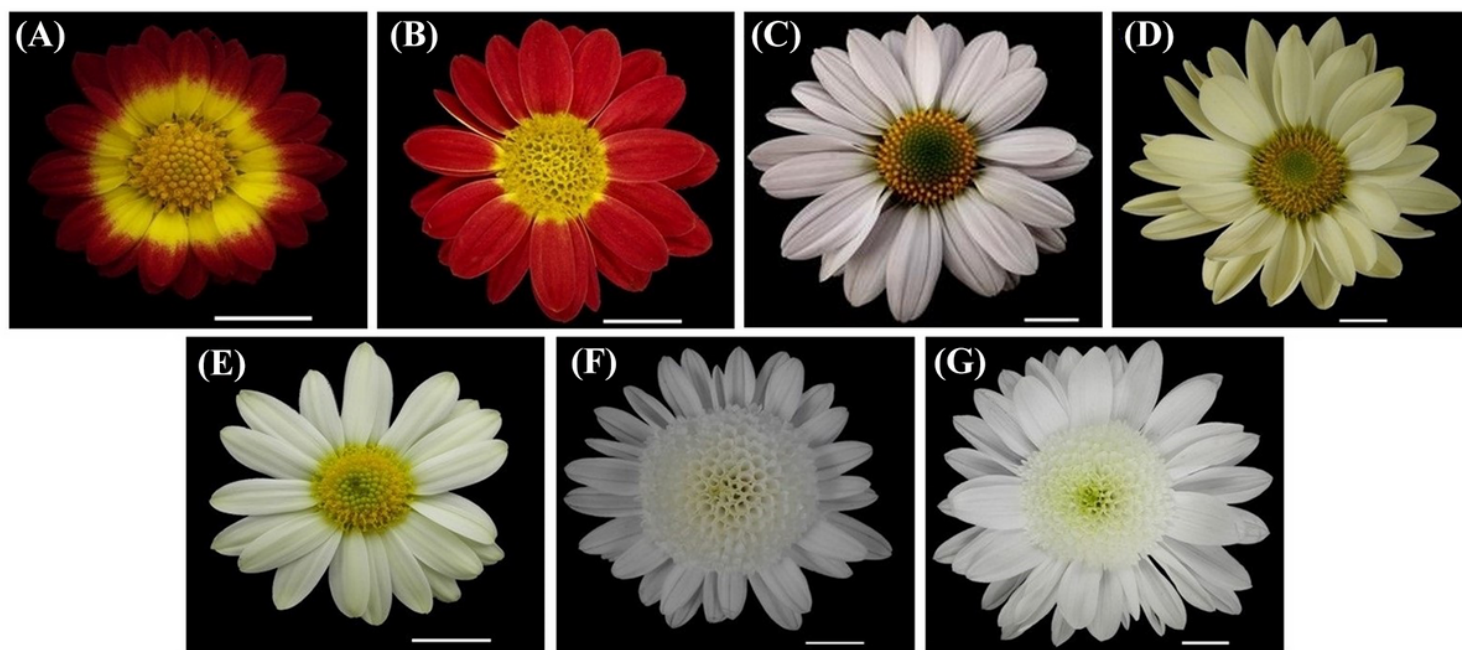


Figure 1

Inflorescence characteristics of seven parental cultivars of chrysanthemum. A-F represents the cut chrysanthemum cultivars, 'QX021', 'QX081', 'QX096', 'QX097', 'QX098', 'Nannong Xuefeng' and 'Monalisa', respectively. Bar = 10 mm

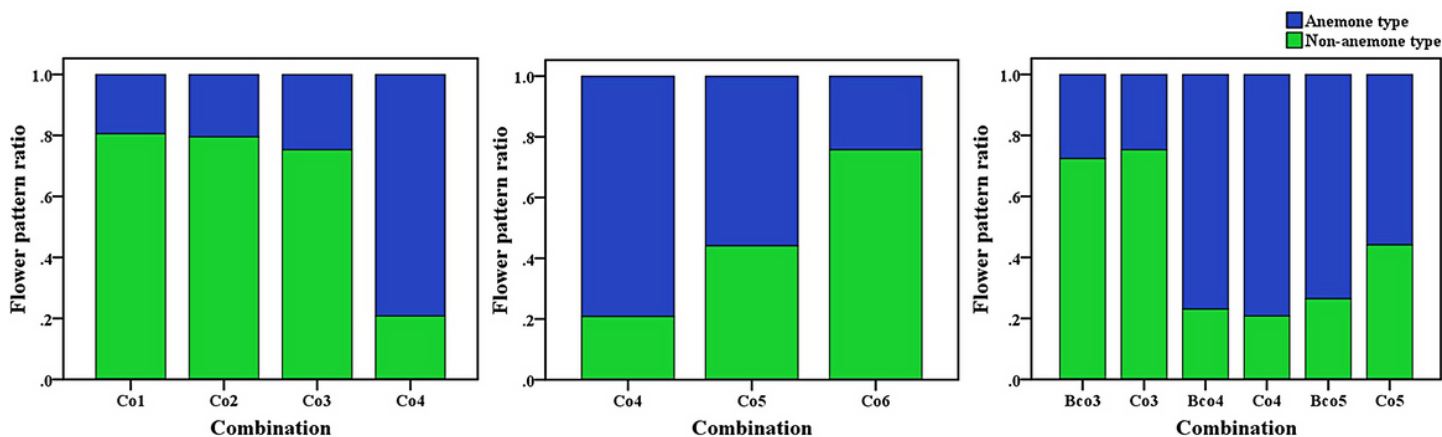


Figure 2

Box-plot showed the genetic variation of six floral traits in the F_1 populations of the same male parent (A) and the female parent (B)

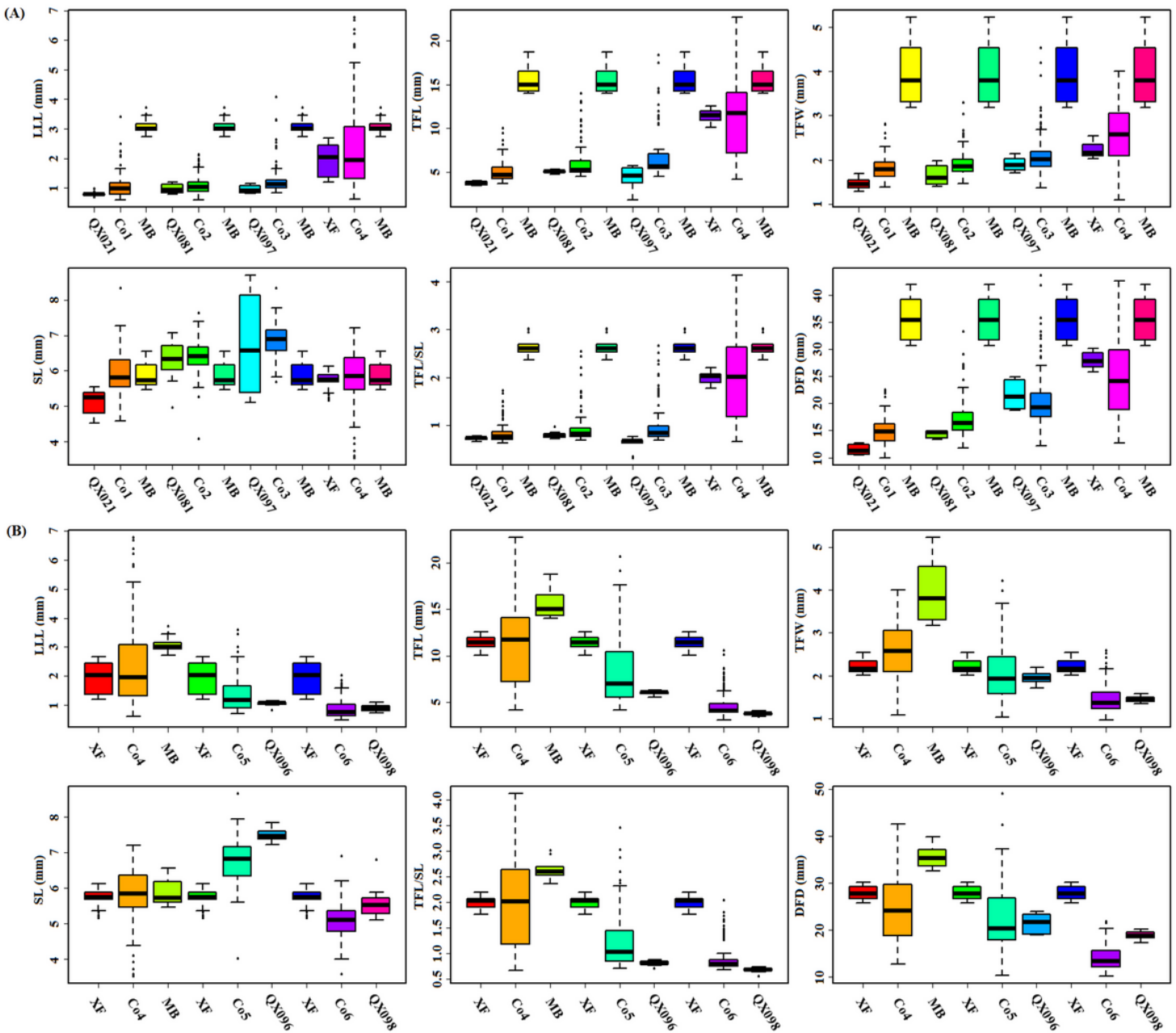


Figure 3

Flower pattern separation ratio in different cross combinations

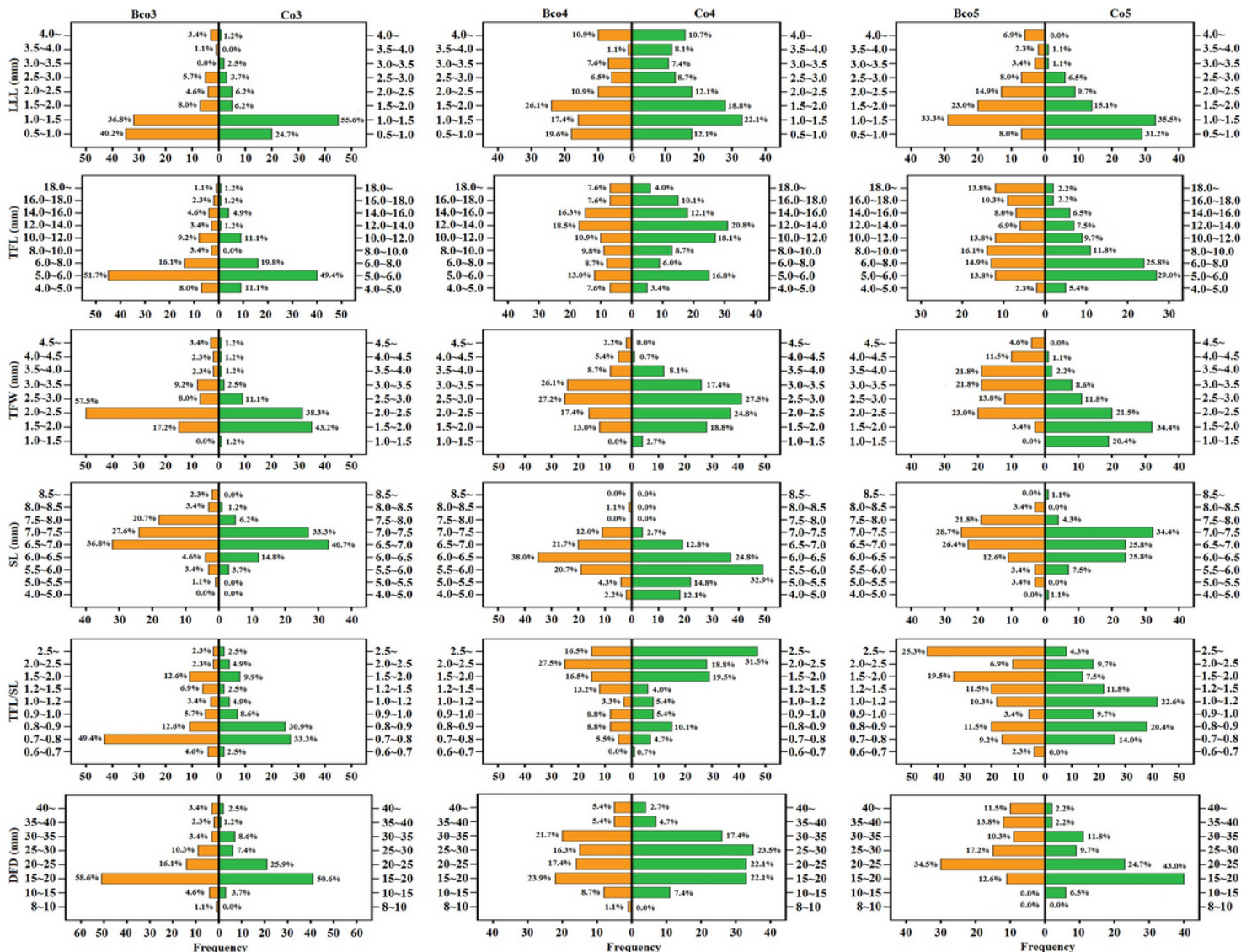


Figure 4

The frequency distribution of six floral traits in three reciprocal crosses

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