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ORIGINAL ARTICLE

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## **Heritability and phenotypic correlation of traits related to N<sub>2</sub>-fixation and agronomic traits in peanut (*Arachis hypogaea* L.)**

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### **Abstract**

Phudенpa, A., Jogloy, S., Toomsan, B., Wongkaew, S., Kesmala, T. and Patanothai, A.

**Heritability and phenotypic correlation of traits related to N<sub>2</sub>-fixation and agronomic traits in peanut (*Arachis hypogaea* L.)**

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Broad-sense heritability is useful information for plant breeders to formulate effective breeding strategies as it provides rough estimates of genetic variability in the breeding materials. The experiment was conducted under field condition in a farmer's farm following rice harvest in Kalasin province in the Northeast, Thailand during January to May 2000. The objective of this study was to estimate broad-sense heritabilities and correlation coefficients between traits related to N<sub>2</sub>-fixation and agronomic traits in the F<sub>2</sub> generation derived from diallel crosses involving 5 parent lines of peanut. Data were recorded for top dry weight, nodule dry weight, leaf color score, pod weight per plant, seed weight per plant, pod number per

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plant, seed number per plant, 100-seed weight and shelling percentage. Zero or near zero heritability estimates were found for leaf color score. High heritability estimates were observed for shelling percentage. Heritability estimates for top dry weight were low to high, ranging from 0 to 0.98. Heritability estimates for nodule dry weight, pod dry weight, seed dry weight, pod number per plant, seed number per plant and 100 seed weight were low to moderate. Top dry weight was positively correlated with nodule dry weight, leaf color score, pod dry weight, seed dry weight, pod weight per plant, seed weight per plant and 100-seed weight, but it had significant negative correlation coefficients with shelling percentage. Pod dry weight, seed dry weight, pod number per plant and seed number per plant were positively correlated with each other.

**Key words :** broad-sense heritability, correlation coefficient

### บทคัดย่อ

อริสรา ภูเด่นพา<sup>1</sup> สนั่น จอกลอย<sup>1</sup> บรรยง ทุมแสน<sup>1</sup> โสภณ วงศ์แก้ว<sup>2</sup> ถวัลย์ เกษมาลา<sup>1</sup>  
และ อรันต์ พัฒโนทัย<sup>1</sup>

อัตราส่วนพันธุกรรมและสหสัมพันธ์ของลักษณะที่เกี่ยวข้องกับการตรึงไนโตรเจนและ  
ลักษณะทางการเกษตรของถั่วถิง

ว. สงขลานครินทร์ วทท. 2547 26(3) : 317-325

อัตราส่วนพันธุกรรมแบบกว้าง เป็นข้อมูลที่มีประโยชน์สำหรับนักปรับปรุงพันธุ์พืช ในการเลือกใช้วิธีการ  
ปรับปรุงพันธุ์อย่างมีประสิทธิภาพ ได้ทำการทดลองในสภาพไร่ในนาเกษตรกรรมหลังเก็บเกี่ยวข้าว ที่จังหวัดกาฬสินธุ์ โดย  
มีวัตถุประสงค์เพื่อประเมินอัตราส่วนพันธุกรรมแบบกว้าง และสัมประสิทธิ์สหสัมพันธ์ ระหว่างลักษณะที่เกี่ยวข้อง  
กับการตรึงไนโตรเจนและลักษณะทางการเกษตร ในประชากรชั้นที่ 2 ของถั่วถิง ที่เกิดจากการผสมข้ามพันธุ์แบบ  
พบกันหมุนของพ่อแม่ 5 พันธุ์ (full diallel) บันทึกข้อมูลลักษณะน้ำหนักต้นแห้ง น้ำหนักปมแห้ง คะแนนสีใน  
น้ำหนักฝัก/ต้น น้ำหนักเมล็ด/ต้น จำนวนฝัก/ต้น จำนวนเมล็ด/ต้น น้ำหนัก 100 เมล็ด และเบอร์เซ็นต์กะเทาะจาก  
ผลการทดลอง พนวณ อัตราพันธุกรรมของลักษณะคะแนนสีในเมล็ด 0 หรือใกล้ 0 อัตราส่วนพันธุกรรมของ  
ลักษณะเบอร์เซ็นต์กะเทาะมีค่าสูงในเกือบทุกคุณสมบัติ อัตราส่วนพันธุกรรมของลักษณะน้ำหนักต้นแห้งมีค่าตั้งแต่ 0 ถึง  
0.98 อัตราส่วนพันธุกรรมของลักษณะน้ำหนักปมแห้ง น้ำหนักฝัก น้ำหนักเมล็ด จำนวนฝัก/ต้น จำนวนเมล็ด/ต้น  
และน้ำหนัก 100 เมล็ด มีค่าต่ำลงมาถึง 0.02 สำหรับลักษณะน้ำหนักปมแห้ง คะแนนสีใน  
น้ำหนักฝัก น้ำหนักเมล็ด จำนวนฝัก/ต้น จำนวนเมล็ด/ต้น และน้ำหนัก 100 เมล็ด แต่มีสหสัมพันธ์ทางลบกับ  
ลักษณะเบอร์เซ็นต์กะเทาะ ส่วนน้ำหนักฝัก น้ำหนักเมล็ด จำนวนฝัก/ต้น จำนวนเมล็ด/ต้น มีสหสัมพันธ์ทางบวก  
ซึ่งกันและกัน

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Modern crop production depends mainly on the application of chemical fertilizers particularly nitrogen fertilizer. This input adds up to the total cost of crop production. Symbiotic nitrogen fixation by leguminous species including peanut offers an alternative way to reduce nitrogen ferti-

lization. Peanut residue also provides nitrogen for following crops such as rice, cassava, sugarcane and field maize. Consequently, the cost of crop production is reduced, soil fertility is recovered and sustainable agriculture can be obtained. The development of peanut lines with high nitrogen

fixing ability is, therefore, the main objective of the peanut breeding program at Khon Kaen University.

Effective selection in early generations of segregating materials can be accomplished only when additive genetic effects are substantial and heritability is high (Phillips *et al.*, 1989a). In generation mean analysis, Phillips *et al.* (1989b) reported that dominance and epistatic gene effects were more important than additive gene effects for number of nodules, top dry weight and nitrogenase activity (acetylene reduction). Anderson *et al.* (1993) reported that non-additive gene effects were significantly important for pod size and seed size, whereas additive gene effects were important for pod length and seed size.

Chiow and Wynne (1983), using parent-offspring regression, found that high heritability estimates were observed for yield and protein content followed by pod length and seed weight but maturity index had low heritability estimates. Arrendell *et al.* (1985) reported that broad-sense heritability estimates for nodule number, nodule weight, nitrogenase activity, top dry weight and pod dry weight of crosses, resulting from NC 9 as a parent were higher than those of crosses involving breeding line 922 as a parent except for specific nitrogenase activity and nitrogenase activity had contribution to top dry weight rather than pod dry weight.

Reports on relationships of agronomic traits indicated that pod yield was related to seed yield and seed size (Hamid *et al.*, 1981; Chiow and Wynne, 1983; Jogloy *et al.*, 1987). Chiow and Wynne (1983) also found that seed size was related to pod length but was not related to shelling percentage.

The accurate methods for determining symbiotic nitrogen fixation are based on <sup>15</sup>N techniques and Kjedahl analysis. These methods are costly and require well-equipped laboratory. Toomsan *et al.* (1991) found that the amount of N<sub>2</sub> fixed per plant had the highest correlation value with total N content in shoot (0.97\*\*\*) followed by shoot dry weight (0.74\*\*), % nitrogen derived from atmosphere (0.67\*\*), nodule

weight (0.60\*\*), root dry weight (0.46\*\*\*) and nodule dry weight (0.45\*\*), suggesting that shoot dry weight could be used as a selection criterion for high nitrogen fixation in where well-equipped laboratory is not available.

Symbiotic nitrogen fixation in peanut has been affected by many factors viz. soil types, soil moisture availability, indigenous rhizobium, peanut genotypes and interaction of these factors. Effective rhizobium strains from greenhouse evaluation may be not competitive with indigenous rhizobium. Nitrogen fixation in peanut by inoculation with effective strains is inconsistent and often unsuccessful (Toomsan *et al.*, 1988). Therefore, screening and selection of peanut genotypes with high nitrogen fixing ability should be conducted in target areas. The objectives of this study were to estimate broad-sense heritabilities and to determine the relationships of traits related to N<sub>2</sub>-fixation and agronomic traits in the F<sub>2</sub> generation of 19 crosses under environmental condition in Thailand for getting information needed for breeding.

## Materials and Methods

### Plant materials and experimental procedures

Three peanut lines, A3-1-1(Bc 154) (PI 268 770), 42-9-105 (PI 269 109) and Virginia bunch (PI 152 133), selected for high top dry weight, number of nodules and nodule weight from previous evaluation (Toomsan *et al.*, 1991), and two high yielding cultivars, KKU 1 and KKU 72-1, were crossed in a diallel fashion with reciprocals to generate 20 F<sub>1</sub> hybrids. KKU 1 is a Spanish-type and KKU 72-1 is a Virginia-type cultivar released by Khon Kaen University. For convenience, A3-1-1 (Bc 154) (PI 268 770), 42-9-105 (PI 269 109) and Virginia bunch (PI 152 133) are hereafter designated as PI 268 770, PI 269 109 and PI 152 133, respectively. F<sub>1</sub> hybrids of 20 crosses were allowed to self-pollinate to produce the ample F<sub>2</sub> seeds for evaluation. However, the cross PI 269 109 x PI 268 770 was not included because of inadequate seeds.

F<sub>2</sub> seeds of 19 crosses (one cross was deleted because of seed shortage.) and their 5 parental lines were evaluated under field condition in a farmer's field following rice harvest planting in Kalasin province in the Northeast, Thailand during January to May 2000. The 24 entries were assigned in a randomized complete block design with 4 replications.

Filter cake (waste from sugar mill) at the rate of 12.5 tons ha<sup>-1</sup> was incorporated into the top layer of soil ridges, which were raised by two-wheel tractor, during soil preparation to reduce soil nitrogen availability. Seeds were planted on soil ridges with 5 m long and 0.8 m wide, which could accommodate 2 peanut rows with spacing of 50 cm between rows and 20 cm between plants within row. Plot size was 4 row plot, accommodated on 2 soil ridges. Seeds were treated with ethephon 0.02 % to break possible dormancy. Soil was inoculated soon after planting with mixed strains of cowpea-type *Rhizobium* to ensure uniform nodulation of peanut plants. Soil inoculation was accomplished by spreading the inoculum (1:10 w/v of *Rhizobium* and water) on the raised beds.

Seeds were over-planted and then seedlings were thinned to obtain 1 plant per hill at 15 days after planting. Mechanical weeding was done at 20 days after planting. Chemical fertilizer at the rate of 0-56.25-37.5 kg ha<sup>-1</sup> of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O was applied soon after weeding. Gypsum (CaSO<sub>4</sub>) at rate of 312.5 kg ha<sup>-1</sup> was applied at 40 days after planting. At the same time, carbofuran 3 % G at the rate of 31.25 kg ha<sup>-1</sup> was used to prevent pod damage from subterranean ants (*Dorylus orientalis* West). Furrow irrigation was available as needed. Harvesting time was determined by pod scrape technique as suggested by Williams and Drexler (1981).

### Data collection

Data were collected based on individual plants in the plot for top dry weight, nodule dry weight, leaf color, pod weight per plant, seed weight per plant, pod number per plant, seed number per plant, 100 seed weight and shelling percentage.

Leaf color was observed at 50 days after planting by scoring 1=pale yellow, 2=yellow, 3=light green, and 4=dark green on each plant in the plot. Other traits were recorded at harvest. At maturity, plants were dug by hand-hoe. Shoots, nodules and pods were taken from each plant and kept in separated paper bags. Nodules were oven-dried at 75° C for 48 hours and weighted to obtain nodule dry weight. Shoots were oven-dried at 75° C for 48 hours and weighed to obtain top dry weight. Pods were air-dried to obtain approximately 8 % moisture content, shelled and weighted. Then, pod number per plant, pod weight per plant, seed weight per plant, seed number per plant, 100-seed weight, shelling percentage and harvest index were determined.

### Data analysis

**Heritability-** Broad-sense heritability (h<sup>2</sup><sub>bs</sub>) estimates of each cross based on individual plants in the plot were calculated for traits related to N<sub>2</sub>-fixation and agronomic traits, using the relationships as follows: -

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad [\text{Eq. 1}]$$

$$h_{bs}^2 = \sigma_g^2 / \sigma_p^2 + \sigma_e^2 \quad [\text{Eq. 2}]$$

$$h_{bs}^2 = [\sigma_{F2}^2 - (\sigma_{P1}^2 + \sigma_{P2}^2)/2] / \sigma_{F2}^2 \quad [\text{Eq. 3}]$$

where:  $\sigma_{F2}^2$  = The variance of any cross =  $\sigma_p^2$

$\sigma_{P1}^2$  = The variance of female parent

$\sigma_{P2}^2$  = The variance of male parent

Estimate of environmental variance ( $\sigma_e^2$ ) for any cross was calculated as  $(\sigma_{P1}^2 + \sigma_{P2}^2)/2$ . In each cross, variances between plants of the F<sub>2</sub> and parents were obtained from the analysis of variance following a completely randomized design, assigning replications as classes. Therefore, variation within classes is the variation among plants of each generation as shown in Table 1. Total variation of each generation, after elimination of among class variance, was used for estimation of genotypic or phenotypic variation of the generation and the heritabilities.

**Table 1. Analysis of variance of crosses and parents.**

Sources of df variation	MS		
	$F_2$	$P_1$	$P_2$
Among classes t-1 (block)	MST	MST	MST
Among plants t(r-1) within class	MSE = variance $F_2$	MSE = variance $P_1$	MSE = variance $P_2$

t = Number of replications of each entry. ; r = Number of plants within replication.

**Phenotypic correlation-** Simple phenotypic correlation coefficients (based on plot means of 19  $F_2$  crosses) among  $N_2$ -fixation and related traits were calculated, using correlation function of MSTAT-C (Bricker, 1989).

## Results and Discussion

### Heritability

Broad-sense heritability for traits related to  $N_2$ -fixation, e.g. top dry weight, nodule dry weight and leaf color score, and agronomic traits were evaluated in the  $F_2$  population. Parental lines were significantly different for top dry weight, leaf color score, pod weight per plant, seed weight per plant, 100-seed weight and shelling percentage, but not significant for nodule dry weight, pod number per plant, and seed number per plant (data not reported), indicated sufficient divergence for the traits under study.

**Traits related to  $N_2$ -fixation-** The heritability estimates for all traits related to  $N_2$  fixation were low, especially for leaf color score in which the heritability estimates were zero or near zero in most crosses (Table 2). Negative heritability estimates were expected to be zero and expressed as zero values. Heritability estimates for nodule dry weight were low to moderate, ranging from 0 to 0.67. However, high heritability estimates were found for top dry weight in the crosses PI 269 109 x PI 152 133 (0.98), PI 152 133 x PI 269 109 (0.94) and PI 268 770 x PI 269 109 (0.90).

**Agronomic traits-** High heritability estimates were found for shelling percentage in most crosses. The heritability estimates for the rest of agronomic traits varied from zero to high, depend-

ing on traits and crosses. Heritability estimates for pod dry weight, seed dry weight, pod number per plant, seed number per plant and 100-seed weight were also low to moderate, ranging from 0 to 0.67, 0 to 0.55, 0 to 0.80, 0 to 0.57 and 0 to 0.76, respectively. Very few crosses with moderate heritability estimates were found in these traits. Selection for individual traits will be difficult since genetic variation is low.

Those partly mentioned results were in agreement with those of Arrendell *et al.* (1985), who reported that the heritability estimates for top dry weight, nodule number, nodule weight, nitrogenase activity and pod weight per plant were high. In contrast, Miller *et al.* (1986) reported that the heritability estimates for nodule weight and top dry weight were low. The discrepancy of the results is not unexpected because such quantitative traits are often affected by several environmental factors. Assessment at different times may be the main cause of differences in the results (during growth phase and at harvest). Peanuts are also different in maturity, disease resistance and tolerance to different kinds of stress. Foliar diseases can cause leaf defoliation that affects top dry weight, nodulation, pod yield and seed yield. Severe *Peanut bud necrosis virus* (PBNV) infection in our experimental field could affect leaf color score assessment and other traits.

Considering the low heritability estimates for most traits, the improvement of these traits in the  $F_2$  generation by selecting individual plants will not be possible. Although there were high heritability estimates for shelling percentage in most crosses, they did not provide an indication for genetic advance through selection because

Table 2. Heritability estimates for traits related to N<sub>2</sub>-fixation and agronomic traits in the F<sub>2</sub> generation of 19 crosses of peanut.

Cross	Traits related to N <sub>2</sub> -fixation			Agronomic traits					
	Top dry weight	Nodule dry weight	Leaf color score <sup>u</sup>	Pod dry weight	Seed dry weight	Pod number/ plant	Seed number/ plant	100 seed weight	Shelling percentage
KKU 1 x KKU 72-1	0	0	0	0	0	0	0	0.22	0.86
KKU 1 x PI 152 133	0	0	0	0	0	0	0	0.47	0.97
KKU 1 x PI 269 109	0.77	0.47	0.26	0.05	0	0.13	0	0	0.83
KKU 1 x PI 268 770	0.53	0.17	0	0.59	0	0.26	0	0.41	0.96
KKU 72-1 x KKU 1	0	0	0	0	0	0.05	0.13	0.31	0.90
KKU 72-1 x PI 269 109	0	0	0	0	0	0	0	0	0.75
KKU 72-1 x PI 268 770	0	0	0	0	0	0	0	0.55	0.85
KKU 72-1 x PI 152 133	0.08	0	0	0	0	0.33	0	0.76	0.89
PI 269 109 x KKU 72-1	0.17	0	0	0.45	0.38	0.43	0.46	0.17	0.91
PI 269 109 x KKU 1	0.79	0.27	0	0.01	0	0	0.24	0.17	0.98
PI 269 109 x PI 152 133	0.98	0.34	0	0.41	0	0.24	0.32	0	0.90
PI 152 133 x KKU 72-1	0	0	0	0	0	0	0	0	0.79
PI 152 133 x KKU 1	0	0	0	0	0.17	0	0	0.18	0.90
PI 152 133 x PI 269 109	0.94	0.67	0	0.45	0.18	0.19	0.21	0	0.95
PI 152 133 x PI 268 770	0.89	0.23	0	0.21	0.12	0.80	0.57	0.24	0.96
PI 268 770 x PI 152 133	0.83	0	0	0	0	0	0	0.32	0.91
PI 268 770 x KKU 1	0.48	0.01	0	0.36	0	0	0	0.21	0.91
PI 268 770 x PI 269 109	0.90	0.59	0	0.67	0.55	0.65	0.58	0	0.86
PI 268 770 x KKU 72-1	0	0.39	0	0	0	0	0	0.31	0.82

Remark: Negative heritability estimates were expected to be zero and expressed as zero values.

<sup>u</sup> Score 1-4 where 1= pale yellow 4= dark green.

only narrow sense heritability is included in the genetic gain equation. Therefore, selection should be conducted in the advanced generations of selfing when the breeding materials can be duplicated for extensive evaluation. Then, selection based on family performance or progeny test should be more effective.

### Phenotypic correlation

Correlation coefficients between traits related to N<sub>2</sub>-fixation and agronomic traits were evaluated in the F<sub>2</sub> generation based on plot means of 19 crosses. Highly significant positive correlation coefficients were found for top dry weight with nodule dry weight (0.55\*\*) and top dry weight with leaf color score (0.45\*\*), but nodule dry weight had weak correlation with leaf color score (0.29\*) (Table 3). Top dry weight also had highly significant positive correlation coefficient with seed dry weight (0.88\*\*), pod number per plant (0.59\*\*), seed number per plant (0.45\*\*) and 100-seed weight (0.54\*\*), but had negative correlation coefficient with shelling percentage (-0.43\*\*). The relationships between nodule dry weight and agronomic traits were not strong, indicating the independent segregation for these traits or the less contribution of N<sub>2</sub>-fixation to agronomic performance. Leaf color score had low correlation coefficients with pod dry weight (0.30\*\*), seed dry weight (0.22), pod number per plant (0.21), seed number per plant (0.17) and 100-

seed weight (0.30\*\*), but had highly significant negative correlation coefficient with shelling percentage (-0.61\*\*).

The results were in agreement with previous studies. Walls *et al.* (1985) reported that top dry weight, nodule dry weight and leaf color score were positively correlated with pod weight per plant, seed weight per plant, pod number per plant, seed number per plant and 100-seed weight. Similarly, Swe and Branch (1986) showed that top dry weight was correlated with pod number per plant and seed number per plant.

In contrast, Arrendell *et al.* (1986) found that selection for increased shoot weight did not increase pod weight, but selection for increased nitrogenase activity could improve pod yield as indicated by mean difference between the two groups selected for high and low nitrogenase activity. They also found that genotypic correlation between nitrogenase activity and shoot weight was high, indicating that selection for shoot dry weight may be an effective means of indirect selection for acetylene reduction and thus for yield, but may be in lesser extent.

Pod dry weight, seed dry weight, pod number per plant and seed number per plant were closely correlated with each other. Pod dry weight and pod number per plant were also significantly correlated with 100-seed weight with the coefficient values of 0.47\*\* and 0.35\*\*, respectively. Pod dry weight, seed dry weight, pod number per plant, seed

**Table 3. Phenotypic correlation coefficients for traits related to N<sub>2</sub>-fixation and related traits in the F<sub>2</sub> generation of 19 crosses of peanut.**

	Nodule dry weight	Leaf color score	Pod dry weight	Seed dry weight	Pod number/ plant	Seed number/ plant	100 seed weight	Shelling percentage
Top dry weight	0.55**	0.45**	0.49**	0.58**	0.59**	0.45**	0.54**	-0.43**
Nodule dry weight		0.29*	0.06	0.28*	0.25*	0.17	0.26*	-0.24*
Leaf color score			0.30**	0.22	0.21	0.14	0.30**	-0.61**
Pod dry weight				0.67**	0.66**	0.43**	0.47**	-0.25*
Seed dry weight					0.84**	0.66**	0.21	0.01
Pod number/plant						0.66**	0.35**	-0.14
Seed number/plant								0.06
100 seed weight								-0.18

\*,\*\* Significant at 0.05 and 0.01 probability levels, respectively.

number per plant and 100-seed weight had low correlation coefficient with shelling percentage.

For agronomic traits, Jogloy *et al.* (1987) and Hamid *et al.* (1981) found that pod weight per plant and seed weight per plant were correlated with each other, and shelling percentage also had positive correlation with harvest index. Recently, Kesmala (2003) found that pod weight per plant, seed weight per plant and pod number per plant were closely correlated with each other and relationships of these traits with 100-seed weight were significantly positive, while relationship between 100-seed weight and shelling percentage was not significant. Relationships among agronomic traits from several studies were inconsistent, presumably due to differences in materials used. For example, small seed size is often associated with high shelling percentage and harvest index because of the thinner shells. Based on the results, selection for individual traits may be more effective than selection for multiple traits. In the traits with low heritabilities, selection should be conducted in later generations of inbreeding based on family mean or progeny test rather than individual plants to reduce large environmental effects. Large environmental effects and variety x year interaction for traits indicative of nitrogen fixation and agronomic traits were also detected in the parental lines (Jogloy, unpublished results). Even though genetic variability exists, the environmental noise is also large. This may cause low heritability estimates for most traits in some crosses under study.

### Conclusion

The results of this study were restricted to one location experiment due to the limitation of F<sub>2</sub> seed availability. Under field condition where environmental variation was difficult to control, heritability estimates based on individual plants were low for nodule dry weight, pod dry weight, seed dry weight, pod number per plant, seed number per plant and 100-seed weight. High estimates were found for shelling percentage only and in some crosses for top dry weight. Considering relationships among traits under study, weak asso-

ciation among traits indicates that selection for individual traits may be more effective than that for multiple traits. For traits with low heritability estimates, selection should be practiced in later generations of inbreeding when duplicate materials are available for extensive testing.

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