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Argenteum (*Arg*) mutant of *Pisum*

Genetic control and breeding behavior

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ABSTRACT: Pea plants bearing the dominant gene argenteum (*Arg*) have gray-green, silvery foliage that contrasts sharply with the green of normal (*arg*) plants. The mutant phenotype results from extensive air spaces beneath the leaf epidermis. *Arg* segregates in a Mendelian manner and is linked with *Pl* and other markers on chromosome 6. The phenotype, however, is not completely stable. Instability is marked by variegation—irregular patches of green tissue—and by distorted segregation ratios. Distorted ratios result from a higher than expected number of green offspring, the excess being attributable to plants that mimic those produced by Mendelian segregation but originating in a non-Mendelian manner. The marker, *Pl*, aids in distinguishing such green plants, designated "arg", from normal *arg*/*arg* segregants. Green "arg" plants also may descend directly from *Arg*/*Arg* plants. *Arg*/*Arg* plants that are variegated in the reproductive region produce more "arg" offspring than those that are variegated in the vegetative region only, or those that are not variegated (visually) at all. Once the *Arg* phenotype is converted to "arg" the latter is inherited as monogenic recessive. Phenotypic instability could not be satisfactorily explained by any of the mechanisms investigated, including nuclear/cytoplasm interaction and modifier gene action. An hypothesis invoking high mutability would require simultaneous or sequential mutation at both *Arg* alleles of *Arg*/*Arg* plants. Moreover, since "arg" plants can be induced to develop a near-mutant phenotype upon exposure to high growth temperature, this implies that "arg" is not a back mutation to *arg*. Thus, the distinction between tissue specific gene action and gene change is still unclear.

THE ARGENTEUM (*Arg*) mutant of the garden pea (*Pisum sativum* L.), first isolated by L. G. Cruger, has distinctive phenotypic and histological features[4] as well as an unusual breeding behavior[9]. Mutant plants have a conspicuous gray-green, silvery cast that is evident from the early seedling stage until senescence. There are no other obvious differences between mutant and normal plants, and the fecundity of both phenotypes appears equal. Hoch et al.[4] ascribed the mutant phenotype to the presence of extensive air spaces underlying the epidermal layers of the leaflets. The epidermis of *Arg* plants is weakly attached, allowing it to be peeled in large sheets from the adaxial and abaxial leaf surfaces with little or no tearing of the subjacent cell layers. The mutant therefore may play a useful role in certain physiological studies, such as those involving stomatal behavior[5], where a single intact layer of cells is desired.

Sporadically, *Arg* plants become variegated, exhibiting a highly irregular mix of mutant (gray-green) and normal (green) tissue. Variegation, in turn, evidently is associated with anomalous breeding behavior. Although examples of variegation abound[3,6], the specific type of variegation in the *Arg* mutant is uncommon and its nature is still enigmatic. Pearson[12] discussed several examples of phenotypic instability in plants and considered various mechanisms that might account for such behavior.

Evidence presented here shows that the argenteum phenotype is transmitted in a Mendelian manner. The phenotypic instability that characterizes this mutant, as well as the results of attempts to probe the nature of the phenomenon, are described.

Materials and Methods

Experimental plants were cultivated in the field, greenhouse, and in growth chambers. In the field, seed were planted about 7 cm apart in rows and the plants were supported by wire trellises, permitting the plants to be examined and scored on an individual basis. Mature, dry

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seed was harvested, identified, and handled on a single-plant basis.

In the greenhouse, plants were grown individually in 1-quart plastic pots filled with Cornell Mix[1]; they were watered, fertilized, and trained according to procedures that assured good growth. Some plant populations also were grown in flats (63 plants per flat) containing builders' or quartz sand after which some individual plants were transplanted to pots and grown to maturity. Greenhouse controls were set to achieve temperatures of 20°C day/15°C night. Natural daylength was supplemented to 16 hours by light from VHO fluorescent tubes and 200 watt incandescent bulbs.

In exploratory experiments conducted to test the influence of growth environment on phenotypic stability, greenhouse conditions were used to provide near-optimal growth environment while growth cabinets were used to provide conditions approaching the upper and lower limits for satisfactory growth. Two growth cabinets were maintained at 10°C constant and two at 30°C constant. One of the two cabinets at each temperature regime was set to provide high (ca. 95 percent) and the other low (ca. 50 percent) relative humidity. Constant light was provided in each cabinet by fluorescent tubes and incandescent bulbs, giving a photosynthetic photon flux density of ca. 200 $Em^{-2} s^{-1}$ as measured by a LI-COR Quantum meter. Two different lines were exposed simultaneously to these conditions. An F_5 homozygous for *Arg* and *Pl* and presumed to be stable for mutant expression was planted in 10 greenhouse flats, two flats for each environment. Plants were examined for frequency and degree of variegation and for overall phenotypic expression. The second line, an F_5, descended from a single *Pl/Pl* F_4 plant exhibited a normal (green) phenotype but with moderate flecking. Seed were planted in 1-quart plastic pots filled with quartz sand, three seed per pot and six pots per environmental regime. The plants were fertilized about every 10 days with soluble fertilizer.

Results and Discussion

Generally, F_1 plants from reciprocal crosses between normal green (nonmutant) and gray-green plants were gray-green (mutant). The F_2 populations typically consisted of two discrete classes (Figure 1), with segregation ratios conforming to that expected for monogenic control (Table I). Table I also reveals a close linkage (3-4 percent) between *Arg* and *Pl*, a dominant marker located on chromosome 6. The seed of *Pl* plants have a jet black hilum, whereas the hila of normal (*pl/pl*) seed are unpigmented[14]. Since *Pl* affects the maternal tissue of the seedcoat, hilum color segregates on a plant-to-plant basis. Other markers on chromosome 6 have been shown to be linked with *Arg*[10]. In the course of gathering these routine early findings—indicating that the argenteum phenotype is coded by a single dominant nuclear gene residing on chromosome 6—evidence of exceptional breeding behavior began to accumulate.

FIGURE 1 Gray-green (*Arg*/-) and normal green (*arg/arg*) seedlings in a segregating population.

Some *Arg* progenies contained plants with variegated foliage, i.e., leaflets and stipules with irregular patches or sectors of nonmutant (normal green) tissue on otherwise mutant plants. The amount and distribution of such

Table I. Joint segregation of *Arg* and *Pl* in F_2 populations from 8 coupling phase crosses*

Population identification	*Arg Pl*	*Arg pl*	*arg Pl*	*arg pl*	Total
B278-785-825	568	12	19	183	782
B278-536-604	190	3	5	59	257
B278-325, 329-30	25	0	1	9	35
B280-510-515	110	3	2	36	151
B280-545-556	172	3	3	55	233
C280-206	41	0	2	7	50
C280-222-225	90	0	0	31	121
B281-741-804	741	15	15	242	1013
Total	1937	36	47	622	2642

* With one exception all chi-squares for single-gene segregation were nonsignificant at $P = 0.05$ (*Pl-pl* segregation in population C280-206 was significant at $P = 0.05$ but not at $P = 0.01$). All contingency chi-squares for independent assortment were significant at $P = 0.01$

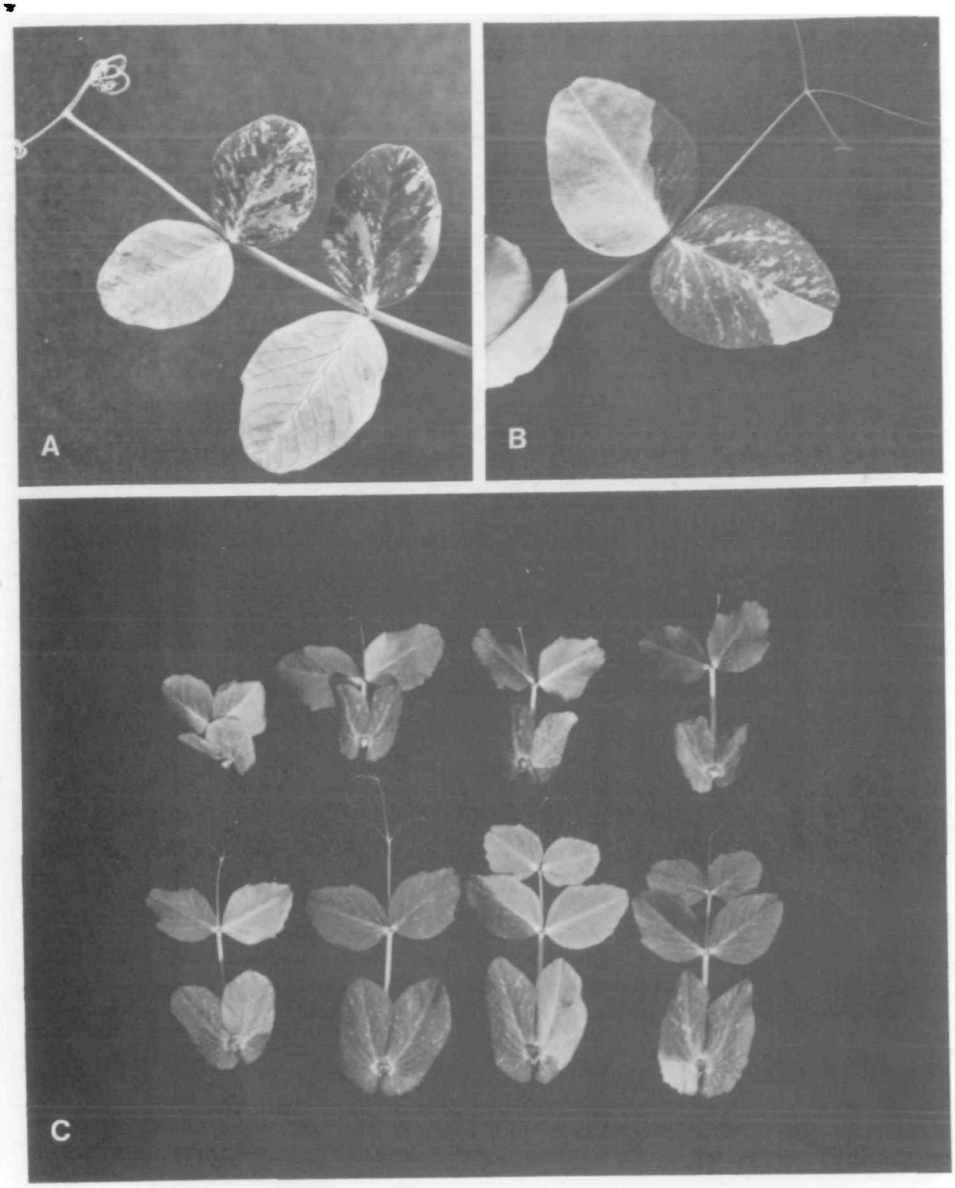

FIGURE 2 *A* and *B* show leaves of different variegated *Arg* plants illustrating absence of fixed pattern. *C*—eight leaves were removed in order of maturity from the same variegated *Arg* plant, with the first formed leaf at top left and last formed leaf at bottom right. Note intervening green (or nearly green) leaves between leaves exhibiting mutant tissue.

patches varied widely from plant to plant and from leaf to leaf on a given plant (Figure 2). The number of variegated *Arg* plants in any given progeny also varied greatly, from none to all. Variegation has been observed in diverse growth environments, in nonsegregating as well as segregating populations, and in every generation examined from F_1 through F_8 (Table II). Green tissue sometimes extended into and included part or all of the pod-bearing region of mutant plants. Occasionally, there was a wholesale conversion of mutant to nonmutant tissue in the early seedling stage so that all the tissue above the first few nodes was green. This irregular, seemingly random, patchwork of mutant and nonmutant tissue resembles many types of chlorophyll variegation[6] except that in this case there is no chlorotic tissue, and it is the mutant phenotype rather than the normal that exhibits the variegation.

Mutant plants were, with rare exceptions, readily distinguishable from normal green plants, even if the former were highly variegated. Whereas most heterozygous gray-green plants (*Arg/arg*) produced progenies in which the number of gray-green and green progeny fit Mendelian expectation, some populations had significantly more green plants than expected (Table II, pops. A378-291, A580-4, A580-5). Since all the green plants in such progenies were phenotypically alike, their origin could not be determined on the basis of phenotype alone. But, by monitoring the marker gene (*Pl*) in populations segregating simultaneously for *Pl* and *Arg*, it was possible to ascertain (except for rare crossover plants) whether a particular nonmutant (green) plant owed its phenotype to normal Mendelian segregation at the *Arg* locus, or to some extraneous cause. The symbol "arg" hereafter is applied to green plants that mimic true *arg* segregants but whose origin was presumed to be non-Mendelian or spurious. Like genuine *arg* segregants, "arg" plants consistently bred true for the green phenotype.

In some instances "arg" plants also appeared in the F_1 generation of crosses between gray-green and green plants. The initial crosses involving the original mutant line from Cruger provided the most extreme example of this. Among 31 F_1 plants grown, 16 were gray-green and 15 were green, a result from which it was incorrectly surmised that the original line was heterozygous (*Arg/arg*) when in fact later evidence showed it to be homozygous dominant. In instances where the mutant parent of crosses was indeed heterozygous (*Arg/arg*), the "arg" plants in the F_1 generation could be distinguished from the true Mendelian segregants by means of the marker gene, *Pl*, just as in the F_2 populations (Table III).

Occasionally, the leaves of "arg" plants displayed somewhat more flecking than usual. Flecking (whitish marbling of the leaves) is common even in normal (*arg/arg*) peas; it varies from line to line and is controlled by the dominant gene *Fl*[13], which is closely linked with *Pl* on chromosome 6[7]. The flecking associated with *Fl* has been attributed to pockets of air space beneath the epidermal layers of leaflets and stipules[2]. Possibly, the phenotype caused by *Arg* is a magnified expression of that caused by *Fl; Arg* and *Fl* therefore may be alleles of the same locus. Allelism tests are in progress but the task is not straightforward. The *Arg* phenotype masks both the *Fl* and *fl* phenotypes, and the unpredictable occurrence of "arg" segregants confuses matters further.

The two manifestations of phenotypic instability in *Arg* populations, viz., variegation on the one hand and anomalous breeding behavior characterized by the presence of "arg" plants on the other, appear to be causally connected. This is best shown in populations descending from mutant plants homozygous for *Arg* inasmuch as *Arg/Arg* plants may be the immediate progenitors of green plants (Tables II and IV). Progenies derived from nonvariegated mutant plants generally produced far fewer "arg" plants than did those from variegated plants (Table IV). Also, seed

collected from mutant plants in which the main stem was nonvariegated produced progeny with fewer "arg" plants than seed collected from variegated branches of the same plant (data not shown). Furthermore, the number of green progeny produced by a homozygous *Arg* plant appeared to be conditional upon the extent to which its reproductive portion was invaded by variegated tissue (Table IV); more "arg" plants occurred in progenies derived from variegated plants in which the variegation extended into the reproductive portion of the plant than those in which no such encroachment was noted. Mutant plants in which the entire reproductive region was converted to green typically produced progeny that were either all "arg" or nearly so. These relationships were not without exception, however. At one extreme, some variegated plants produced progeny all of which were gray-green. Conversely, about 6 percent of the progeny of mutant plants classified as nonvariegated were "arg". These exceptions may trace in part to the fact that the distribution of green tissue on variegated mutant plants was subjectively determined. Plants were assigned into the broad categories shown in Table IV on the basis of visual examination only.

If green tissue must indeed invade the reproductive portion of a mutant plant in order for that plant to produce green plants in its progeny, then presumably the variegated tissue that does not involve the reproductive portion could become isolated and hence not contribute to the production of "arg" plants. Such isolation might result, for example, from the sorting out of causal determinants in a manner akin to the sorting out of chloroplasts in plants exhibiting chlorophyll variegation. However, the reproductive region of a mutant plant may contain so few cells of one tissue type or the other that the differences cannot be detected. Tissue that might appear to be homogeneous or effectively isolated from the reproductive region might in fact be composed of two cell types. Nor was it uncommon for patches of green tissue on variegated mutant plants to be separated by several intervening leaves that were entirely gray-green (Figure 2). Therefore, several architectural features of peas, viz., distichous leaf arrangement, trilacunar nodal anatomy, and the presence of marginal leaf meristems[11], would allow for unpredictable distributions of mutant and nonmutant tissue. Changes are possible at any one of several meristematic sites.

The strong correlative evidence notwithstanding, variegation may not be a precondition for the production of "arg" plants, but instead may be an independent manifestation of phenotypic instability. Even if variegation is a precondition, it is possible that variegation per se rather than variegation in the reproductive region determines the production and frequency of "arg" plants in the ensuing generation. Whatever its mode of origin, phenotypic instability of the character appears to be unidirectional, i.e., from gray-green to green. The phenotypes associated with *Arg* and "arg" are transmitted through pollen and egg alike.

Table II. Distribution of phenotypes in different generations in populations heterozygous (A) and homozygous (B) for *Arg* and *Pl*, each population being derived from single plants in the previous generation. Populations were selected to illustrate phenotypic stability and instability, instability being evidenced by frequency of variegation and by the presence of "arg" plants

Popl. ident.	Gray-green		Green		Total	No. variegated	Generation
	Arg Pl	*Arg pl*	*arg* Pl*	*arg pl*			
A. Heterozygous populations							
A378-202	39	0	0	12	51	17	F_3
A378-291	4	0	16	6	26	0	F_3
A580-4	17	0	58	27	102	17	F_5
A580-5	72	1	13	23	109	29	F_5
B580-351	83	2	1	31	117	4	F_5
B. Homozygous populations							
C378-195	71	0	0	0	71	0	F_3
C579-332	49	0	1	0	50	5	F_5
C579-334	48	0	2	0	50	8	F_5
B580-347	33	0	14	0	47	12	F_5
B580-348	57	0	16	0	73	20	F_5
B580-349	38	0	9	0	47	10	F_5
B580-350	32	0	2	0	34	0	F_5
B580-352	44	0	0	0	44	4	F_5
B580-353	27	0	18	0	45	0	F_5
A680-2	60	0	0	0	60	0	F_6
A680-3	60	0	0	0	60	0	F_6
B780-369	31	0	1	0	32	1	F_7
B780-370	30	0	0	0	30	0	F_7
B780-371	26	0	0	0	26	0	F_7
B780-372	26	0	0	0	26	0	F_7
B780-373	32	0	0	0	32	1	F_7
B780-374	34	0	0	0	34	1	F_7
B780-375	26	0	0	0	26	1	F_7
B881-262	49	0	0	0	49	9	F_8
B881-263	45	0	0	0	45	5	F_8
B881-264	47	0	0	0	47	9	F_8
B881-265	46	0	0	0	46	8	F_8
B881-266	49	0	0	0	49	15	F_8

* Column may contain normal *arg* segregants or "arg" plants

Table III. Distribution of phenotypes in F_1 plants derived from 37 reciprocal crosses between normal green (*arg/arg*) plants with colorless hila (*pl/pl*) and various gray-green (*Arg/–Pl/–*) F_2 segregants from a previous cross

Phenotype of female parent	No. crosses	Gray-green foliage		Green foliage		Total
		Arg Pl	*Arg pl*	*arg* Pl*	*arg pl*	
Gray-green (*Arg/–*) Black hila (*Pl/–*)	15	28	0	8	17	53
Normal green Colorless hila (*pl/pl*)	22	43	1	11	57	112
Total		71	1	19	74	165

* Column may contain normal *arg* segregants or "arg" plants, the latter being of spurious origin

Environmental influence on mutant expression

Growth environment (mainly temperature) had a marked effect on the phenotypic expression of mutant plants. The leaves of *Arg* plants grown at high (ca. 30°C) temperature were strongly crinkled or buckled especially at low relative humidity; crinkling also occurred under greenhouse conditions but to a less striking degree. In some leaves the buckling was so pronounced that epidermal bridges spanned the undulations in the lamina. Little or no buckling of the lamina occurred in the green, nonmutant sectors of variegated *Arg* plants, nor in the leaves of normal green (*arg*) control plants. This suggests that the dynamics of cell expansion in mutant tissue is different from that in normal (*arg*) tissue but that "arg" tissue is similar to *arg* in this respect.

At the low (10°C) growth temperature the characteristic silvery gray-green cast of mutant plants was much less pronounced (more green in hue) than at the high and normal temperature regimes. Crinkling was absent in plants grown at low temperature.

Plants of a selected "arg" line were exposed to the same range of conditions as above in an attempt to induce or restore the mutant phenotype. Although phenotypically nonmutant (green), the "arg" line was regarded as possibly genotypically mutant (*Arg*) because it was a product of non-Mendelian inheritance and because it was rather strongly flecked as well as homozygous for *Pl*. Whereas no phenotypic changes were evident under the normal and low temperature regimes, plants exposed to high temperature and high relative humidity assumed in large part the characteristic gray-green cast of the mutant phenotype (Figure 3). These results were verified in repeat experiments. The treatment had no noticeable effect on control plants (*arg*, *Fl*).

Table IV. Distribution of phenotypes in F_6 progenies derived from individual F_5 plants that were homozygous dominant for *Arg* and *Pl*. F_5 plants were scored for presence or absence of variegation; variegated individuals were divided into categories based on location of involvement. F_6 plants designated *Arg* were gray-green and "arg" plants were green

Main stem				Branches			
no. plants/progeny		no.	total	no. plants/progeny		no.	total
Arg	"arg"	progenies	plants	*Arg*	"arg"	progenies	plants
From non-variegated main stem and branches							
25	0	76	1900	25	0	3	75
24	0	3	72	15	0	1	15
19	0	1	19	16	1	1	17
24	1	2	50	18	7	1	25
23	2	3	75	14	11	1	25
21	4	1	25				
20	5	1	25				
19	6	5	125				
18	7	4	100				
17	8	1	25				
15	10	1	25				
14	11	1	25				
7	6	1	13				
Total		100	2479			7	157
From main stem and branches—variegated, but not in reproductive region							
25	0	6	150	25	0	1	25
10	0	1	10	22	0	1	22
24	1	1	25	19	0	1	19
23	2	2	50	15	0	1	15
22	3	1	25	13	0	1	13
21	4	1	25	10	0	1	10
20	5	1	25	9	0	1	9
18	7	2	50	15	3	1	18
14	7	1	21	10	3	1	13
				18	7	1	25
				0	19	1	19
Total		16	381			11	188
From main stem and branches—variegated in reproductive region							
25	0	2	50	12	3	1	15
22	3	1	25	8	3	1	11
18	6	2	48	6	4	1	10
15	10	2	50	14	11	1	25
14	11	1	25	8	7	1	15
13	12	1	25	4	7	1	11
1	2	1	3	8	17	1	25
6	18	1	24	5	15	1	20
0	3	1	3	6	19	1	25
4	21	1	25	3	12	1	15
0	7	1	7	4	21	1	25
1	24	1	25	1	9	1	10
0	25	1	25	0	11	1	11
0	26	1	26	2	23	1	25
				0	15	1	15
Total		17	361			15	258
From variegated main stem and branches—reproductive region all green							
1	24	1	25	0	20	1	20
0	25	1	25	0	23	1	23
				0	28	1	28
Total		2	50			3	71

Inferences concerning the basis of phenotypic instability

Although this investigation has not yielded an explanation for the variegation and erratic breeding behavior of the *Arg* mutant, some partial insights may be drawn from the following considerations and observations.

Variegation in relation to fertility. The original *Arg* line obtained from Cruger showed reduced seed set. Changes in chromosome structure constitute a potential cause of reduced fertility and such changes are known to be associated with certain kinds of variegation[8]. Although fertility itself was not examined in detail, numerous fully fertile *Arg* lines were selected in the course of these studies. If a relationship between fertility and variegation in *Arg* plants does exist, then it was not apparent in the comparatively large number of lines observed thus far. The regular behavior of the marker gene, *Pl*, may be a further indication that the occurrence of variegation may be independent of fertility.

Heterozygosity. Although the incidence of variegation was observed to be higher in certain populations known to be heterozygous at

the *Arg* locus (*Arg/arg*), no consistent pattern has emerged to implicate heterozygosity as a cause or a triggering mechanism leading to instability. Phenotypic instability has been observed in homozygous *Arg* inbred lines that earlier were believed to be stable.

Mutability of Arg. Certain types of variegation have been ascribed to highly mutable or unstable genes[12]. Since variegation has been observed to occur in lines homozygous for *Arg*, and since "arg" plants may descend directly from such lines, to infer that variegation in the *Arg* mutant is the product of an inordinately high rate of mutation is to suppose that both alleles mutate simultaneously or sequentially from the dominant to the recessive state during the ontogeny of a given individual plant. Moreover, as the temperature experiments showed, it was possible to restore, partially at least, the mutant phenotype in certain green plants suspected on the basis of genetic evidence to possess *Arg*.

Nuclear/extra-nuclear interaction. An attempt was made to determine if stable mutant phenotypic expression depended on an interaction between the nuclear gene, *Arg*, and one or more extra-nuclear elements. The first step in this approach consisted of selecting a number of different *arg/arg pl/pl* (green) segregants from populations in which both *Arg* and *Pl* segregated according to Mendelian expectation and in which variegation was absent. Such plants were considered to be normal ("+") with respect to whatever extra-nuclear factor(s) might be operating. These were used as one parent in a series of reciprocal crosses with selected green plants, dominant for marker *Pl*, derived from populations showing disturbed segregation patterns. Plants of this type were regarded as candidates that lacked some required extra-nuclear element(s) for stability of *Arg* expression. Crosses between these two types of green plant, one *arg pl* ("+") the other *"arg" Pl* ("−"), were designed to determine if complementation between nuclear factors and some putative extra-nuclear factors would result in a restoration of the mutant phenotype in the F_1. The F_1's of 57 such crosses, a total of 233 plants, were observed. None showed typical *Arg* expression. Some plants, however, did appear to be more highly flecked than normal, but not beyond the limits of plants that are recessive for *arg* (*arg/arg*) but dominant for *Fl*. Most of the above plants were carried into the F_4 generation as separate entries without recovering a clear mutant phenotype. Meanwhile, the marker locus showed no deviation from the expected segregation pattern.

Modifier action. To test whether modifier genes might influence phenotypic stability, plants of a F_5 *Arg/Arg Pl/Pl* line stable for *Arg* expression were crossed reciprocally with plants from an unstable line. Parent plants in the stable line were selected from among 50 plants homozygous for *Arg* and *Pl* and showing no evidence of variegation. Parent plants in the unstable line were selected from among variegated gray-green plants in an F_5 that showed the following phenotypic distribution: *Arg Pl*—17; *Arg pl*—0; "arg" *Pl*—58; *arg pl*—27.

Thus the unstable line was heterozygous for *Arg* and for *Pl*. Whereas the marker gene segregated according to expectation (75 *Pl*:27 *pl*), the *Arg* segregation (17 gray-green:85 green) was markedly disturbed. In addition, all 17 of the *Arg*/-plants were variegated. Among four plants in this line used as parents, two were all green and two were initially gray-green but later turned mostly green as a result of considerable variegation. Hence, the latter two crosses may be symbolized as *Arg/Arg Pl/Pl* × *Arg/- Pl/-*.

All 35 of the F_1 plants from these crosses were mutant (gray-green) without any sign of variegation at any time in their ontogeny; all were *Pl/-*. Among 623 F_2 plants descended from the 35 F_1's, 498 were *Arg* (gray-green), only one of which was variegated. Based on the results of the F_2 segregation (Table V), two of the four parental plants—C579-333-(11) and C579-333-(21)—were homozygous for *Pl*, whereas the other two—C579-333-(4) and C579-333-(24)—were heterozygous. Even in the F_2's derived from the parents homozygous for *Pl*, nearly one-fourth (34 of 171, and 16 of 82) of the plants were normal green. Apart from the single variegated F_2 plant, the combined F_2 population displayed only two distinct phenotypes: gray-green and green. Still, the seed of 83 of the green plants had black hila

FIGURE 3 Plants from the same "arg" line grown under optimal conditions in greenhouse (left) and under high temperature (30°C) conditions in growth chamber. Note that the mutant (gray-green) phenotype was nearly restored at 30°C.

(*Pl*). Progenies from these crosses were carried into the F_5 and F_6 generations and were used to show the relationships between variegation and the incidence of "arg" plants summarized in Table IV.

A further series of crosses was made among F_4 plants of the populations just considered. They were made to rule out the unlikely possibility that many of the "arg" plants in the above progenies were actually *arg* plants tracing to an F_2 crossover event. In one set of crosses gray-green plants proven to be homozygous dominant for both *Arg* and *Pl* were crossed with green plants (i.e., "arg") homozygous for the dominant marker *Pl*. Importantly, the latter also derived from progenies that had previously been shown to be *Arg/Arg Pl/Pl*. Another set of crosses was made between different "arg" plants within the group (i.e., "arg" *Pl* × "arg" *Pl*). All 95 F_1 plants from the latter crosses were green. Twenty-seven of these F_1's were tested in F_2 (21 plants per F_2 population, 419 plants in all), and all were green (data not shown).

All 72 F_1 plants derived from the (*Arg Pl* × "arg" *Pl*) reciprocal crosses were gray-green, but many were variegated. Among the 64 F_2 populations deriving therefrom, all but three consisted of gray-green and green plants in ratios approximating 3:1. Two of the exceptional F_2 populations contained green plants exclusively. The third contained gray-green plants exclusively but this population consisted of only 14 plants whereas all other F_2's consisted of 21 plants each. The combined phenotypic distribution resembled monogenic segregation (931 gray-green: 406 green) but with an excess of "arg" plants ($\chi^2_{(3,1)} = 20.5$). It would appear from these and other results that once a plant becomes green, no matter how it originates, "greenness" is inherited as a recessive to gray-green.

Although the crosses considered in this section did not identify specific modifier genes that control phenotypic stability, the observations overall suggest that some lines are more stable than others, due perhaps to some ill-defined differences in background genotype.

Evidence obtained in the preceding section provides useful detail concerning the manner in which the phenotypic alteration is transmitted.

The F_2 phenotypic distribution in Table V reveals that the number of "arg" plants in some F_2 populations (those homozygous for *Pl*) roughly equaled the number of *arg* segregants found in populations segregating in Mendelian manner for both *Arg* and *Pl*. Since, once formed, "arg" behaves as a monogenic recessive factor (Table V), the time that "arg" becomes introduced into the pedigree is key to succeeding events. F_2's derived from crosses involving C579-333-(21) and C579-333-(24) are particularly informative in this regard. Since both plants were gray-green in the seedling stage, clearly each inherited at least one dominant *Arg* allele. Both, however, became mostly green prior to being used as parents in crosses. The F_2 distribution showed C579-333-(21) evidently to be homozygous for *Pl* (and presumably for *Arg* as well) whereas C579-333-(24) was heterozygous for *Pl*. From this it follows that both *Arg* alleles in the former plant and the single *Arg* allele in the latter had became transmuted to "arg" prior to gametogenesis. As a result, both plants contributed only "arg" gametes, and these,

Table V. Distribution of phenotypes in the F_2 from crosses between gray-green plants homozygous for *Arg* and *Pl* ("stable" line) and "arg" plants or *Arg* plants in which the reproductive portion was green (unstable line)

Ident. no. and phenotype of parental plants from unstable line	Popl. ident.	No. plants: gray-green *Arg Pl*	gray-green *Arg pl*	green *arg Pl*	green *arg pl*	Total
	B280-398	10	1	1	2	14
	399	9	0	0	5	14
	400	10	0	0	3	13
C579-333-(4)	402	12	0	0	6	18
Green plant;	Total	41	1	1	16	59
black hilum						
("arg" *Pl*)	B280-396	11	0	2	0	13
	397	11	0	2	0	21
	401	17	0	5	0	22
	403	14	0	0	0	14
	Total	60	0	10	0	70
	B280-385	14	0	5	0	19
	386	17	0	4	0	21
	387	9	0	2	0	11
	388	12	0	1	0	13
C579-333-(11)	389	13	0	3	0	16
Green plant;	390	13	0	3	0	16
black hilum	391	8	0	6	0	14
("arg" *Pl*)	392	8	0	1	0	9
	393	27	0	1	0	28
	394	1	0	6	0	7
	395	15	0	2	0	17
	Total	137	0	34	0	171
C579-333-(21)	B280-411	13	0	3	0	16
Gray-green plant,	412	15	0	1	0	16
turning green;	413	22	0	5	0	27
black hilum	414	16	0	7	0	23
(*Arg Pl*)	Total	66	0	16	0	82
	B280-404	11	0	0	2	13
	407	16	1	1	6	24
	408	10	1	0	5	16
	415	17	0	0	3	20
	416	13	0	0	5	18
C579-333-(24)	419	16	0	1	2	19
Gray-green plant,	Total	83	2	2	23	110
turning green;						
black hilum						
(*Arg Pl*)	405	22	0	5	0	27
	406	20	0	0	0	20
	409	20	0	0	0	20
	410	21	0	0	0	21
	417	14	0	6	0	20
	419	14	0	9	0	23
	Total	111	0	20	0	131

* Column may contain either *arg* segregants or "arg" plants

when paired with the *Arg Pl* gametes from the other parent, produced the observed segregation for gray-green and green phenotypes in F_2, some (the "arg") associated with *Pl* and some (*arg* segregants) with *pl*. The frequency of "arg" plants introduced apparently depends on the degree to which a given *Arg* source plant is converted to green tissue and presumably thereby to "arg" gametes. The conversion may be partial or complete. Thus, virtually any phenotype distribution is possible, including those that fit a 3:1.

This mostly descriptive account leaves unanswered the very questions inspired by the observations themselves. At the core of the matter is the abrupt, unpredictable alteration of the mutant phenotype and the capacity to transmit the change to the offspring.

Differential growth patterns that set up stresses and strains among cells or tissues are commonly cited as the basis for the formation of subepidermal air spaces[3]. The findings of Hoch et al.[4] suggested that the middle lamella between epidermal and palisade cells in leaves of *Arg* may be weaker than the middle lamellae of nonmutant plants. Hara[3] noted that the middle lamella is sufficiently plastic to be separated by growth strains and that this leads to the formation of subepidermal air blisters. The striking crinkling of the leaves of *Arg* plants grown at high temperature may be evidence of even more exaggerated differential growth. The magnitude of the crinkling might imply that the stresses and strains among cells are not confined to the epidermal and subjacent layers, but also may involve differential growth among the mesophyll cells themselves.

Phenotypic instability might well be expected if *Arg* acts by altering the gross relationship between derivatives of the L-I (epidermal) and L-II and L-III layers, since the balance between and among leaf cells and cell type is subject to modification by a variety of internal and external factors. What is not expected, however, is that the changes in the *Arg* phenotype are capable of being transmitted sexually, at least in the manner described in this report. Particularly unclear is whether the sudden, seemingly random and often massive conversion from gray-green to green tissue reflects a permanent genic change at the DNA level that eventually enters the germ line, or instead, reflects a tissue-specific change in gene action. There is some evidence of both. The phenotypic similarity between "arg" and *arg* plants together with certain aspects of breeding behavior suggest that *Arg* may back mutate to *arg*. Yet, the temperature experiments imply that "arg" and *arg* are not equivalent, suggesting in turn that "arg" represents a change in gene action only.

Another possibility is that "arg" represents a back mutation to a new mutational state. Alternatively, if *Arg* is an allele of *Fl*, then "arg" may be a manifestation of *Fl*, the latter having back mutated from a more dominant form, e.g., *Fl Arg*. In the temperature experiments, however, the phenotype of *Fl* plants remained unaltered.

None of the instability phenomena discussed by Pearson[12] or others appears to match in every essential detail the phenomenon described here. Further investigations, especially those conducted at the cell and tissue level of organization, are needed. It is hoped that the present study provides sufficient background to inspire such studies.

References

1. BOODLEY, J. W. and R. SHELDRAKE, JR. Cornell peat-lite mixes for commercial plant growing. Cornell Inf. Bull. 43. New York State College of Agriculture and Life Sciences, Ithaca, NY. 1977.
2. FEDETOV, V. S. Multiple allelomorphs of the character "grey spotting" on the foliage of peas. *Bull. Appl. Bot. Gen. Pl. Breed. Series II* 9:275-286. (In Russian with English Summary). 1935.
3. HARA, N. Study of the variegated leaves, with special reference to those caused by air spaces. *Jap. J. Bot.* 16:86-101. 1957.
4. HOCH, H. C., C. PRATT, and G. A. MARX. Epidermal air spaces: basis for the phenotypic expression of the argenteum mutant of *Pisum*. *Am. J. Botany* 67:905-911. 1980.
5. JEWER, P. C., L. D. INCOLL, and J. SHAW. Stomatal responses of *Argenteum*—a mutant of *Pisum sativum* L. with readily detachable leaf epidermis. *Planta* 155:146-153. 1982.
6. KIRK, J. T. O. and R. A. E. TILNEY-BASSETT. The Plastids. W. H. Freeman, San Francisco. 1967.
7. LAMPRECHT, H. The variation of linkage and the course of crossingover. *Agri Hortique Genet.* 6: 10-48. 1948.
8. LEWIS, E. B. The phenomenon of position effect. *Adv. Genet.* 3:73-115. 1950.
9. MARX, G. A. Argenteum: a mutant under nuclear and extra nuclear control. *Pisum Newsl.* 10:34-37. 1978.
10. ——. The linkage relations of a newly isolated *nana* mutant. *Pisum. Newsl.* 13:35-37. 1981.
11. PATE, J. S. *In* Crop Physiology: Some Case Histories. L. T. Evans, Ed. Cambridge University Press. p. 191-224. 1974.
12. PEARSON, O. H. Unstable gene systems in vegetable crops and implications for selection. *HortScience* 3:271-274. 1968.
13. TEDIN, H. and O. TEDIN. Contributions to the genetics of *Pisum*. IV. Leaf axil colour and grey spotting on the leaves. *Hereditas* 7:102-108. 1925.
14. WHITE, O. E. Studies of inheritance in *Pisum*. II. The present state of knowledge of heredity and variation in peas. *Proc. Am. Phil Soc.* 56:487-588. 1917.