color is recessive to normal green, which agrees with many other previous reports for chlorophyll-deficient mutants found in peanut (Hammons 1973; Murthy and Reddy 1993; Wynne and Coffelt 1982).

The F_2 segregation from each cross fit a 15 normal green:1 Lutescent-Leaf color ratio (Table 1). No significant differences were detected among crosses or between reciprocal crosses, which suggests the absence of cytoplasmic or maternal effects. Total, pooled, and homogeneity chi-squared values also fit a 15:1 ratio. These results suggest that two duplicate recessive genes control the Lutescent-Leaf color trait.

Individual F_2 plant selections were made within two cross combinations (Lutescent-Leaf × Starr and Lutescent-Leaf × NC 7) for subsequent progeny row testing in the F_3 generation. F_3 progeny from F_2 plants with Lutescent-Leaf color bred true to type. Segregation of $F_{2:3}$ progeny from F_2 plants with normal green leaf color fit a 7 nonsegregating (all normal green):4 segregating (15 normal:1 Lutescent):4 segregating (3 normal:1 Lutescent) expected ratio (Table 2). These F_3 results verify the F_2 findings for digenic inheritance.

The data from this genetic study indicate that two recessive genes control the Lutescent-Leaf color trait. The symbols lut_1 and lut_2 are proposed for the genes controlling this Lutescent-Leaf color trait recently found in the cultivated peanut.

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Genetics of a New Male-Sterility Locus in Pigeonpea (*Cajanus cajan* [L.] Millsp.)

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A natural male-sterile mutant was found in the population of a short-duration pigeonpea (*Cajanus cajan* [L.] Millsp.) cultivar ICPL 85010. This mutant is characterized by light yellow anthers of reduced size that are devoid of pollen grains. This mutant was crossed with two pigeonpea cultivars to study its inheritance. The F_1, F_2, and test cross data of the two crosses suggested that this male sterility trait is genetic in origin and is controlled by a single recessive gene. The F_1 (mutant × ICPL 85010) plants were crossed with translucent (ms_1) and arrowhead type (ms_2) genetic male steriles reported earlier to study their allelic relationships. Segregation in the three-way cross F_1 and F_2 populations revealed that the mutant male-sterile gene was nonallelic to ms_1 and ms_2 loci and it is designated ms_3. The new male sterility sources in pigeonpea will help in producing high-yielding hybrids and populations in diverse phenological groups.

Pigeonpea is a short-lived perennial legume shrub. It is mainly cultivated for its dry seeds and green vegetable in dry areas of the tropics and subtropics. In comparison to other food legumes, the pigeonpea plants differ grossly especially with respect to their pollination behavior. The bright colors of pigeonpea flowers attract a number of insects, which affect cross-fertilization with an average natural outcrossing of up to 20% (Saxena et al. 1990). This phenomenon is primarily responsible for the deterioration of varietal purity in this crop. Pigeonpea breeders, however, are using natural outcrossing for the genetic improvement of populations (Byth et al. 1981) and breeding hybrids (Saxena et al. 1996). So far two sources of male sterility have been identified for use in breeding programs. They include a translucent anther type (Reddy et al. 1978) and another with arrowhead-shaped anthers (Saxena et al. 1983). These two male steriles are morphologically distinct and controlled by nonallelic recessive alleles at a single locus, designated as ms_1 and ms_2, respectively.

In the 1997 rainy season, a natural mutant plant completely devoid of pollen grains was identified in the population of the short-duration determinate cultivar ICPL 85010. A close examination of its flowers revealed that the anthers of this mutant were morphologically different from the male-sterility sources reported earlier and were characterized by light yellow color and reduced size. The objective of this article is to report the inheritance of the new male-sterile mutant and its allelic relationship with translucent (ms_1) and arrowhead-shaped (ms_2) male-sterile sources.

Materials and Methods

The mutant plant was crossed with eight randomly selected plants from the same plot for its maintenance, but of these, only one crossed progeny segregated for male sterility. To study the genetics of this trait, the male-sterile segregants of this progeny were crossed with cultivars ICPL 88039 and ICPL 85010. The F_1 plants were used for producing F_2 and testcross populations. To study the genetic relationship of the mutant with ms_1 and ms_2 loci, the F_1 (mutant × ICPL 85010) plants were crossed with other male-sterile lines—MS Prabhat carrying ms_1 allele and MS QPL2 carrying ms_2 allele. The triple cross F_1 plants were selfed for generation advance, and their F_2 progenies were grown in 1999 to study the segregation pattern of different male-sterility loci. The data from different crosses were subjected to chi-squared analysis.

Results and Discussion

Inheritance of the Male-Sterile Mutant

In the F_1 generation all the plants in both the single crosses were fertile. Of the 231 plants grown in the F_2 generation of the mutant × ICPL 85010 cross, 171 were fertile and 60 sterile (Table 1), fitting a 3:1 ratio ($P < .8$). In the other cross (mutant × ICPL 88039), 85 F_2 plants were fertile and 38 sterile, showing a good fit to a 3:1 ratio ($P < .5$). The pooled data from the two crosses also exhibited a good fit to a 3:1 ratio ($P < .8$). The testcross populations of the two crosses and their pooled data fit a 1:1 ratio ($P < .2$), confirming that

Table 1. Segregation for male sterility in F_2 and testcross progenies of two crosses involving male-sterile mutants and cultivars

	Cross					
	Mutant × ICPL 85010		Mutant × ICPL 88039		Pooled data	
Generation	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
F_1	27	0	11	0	38	0
F_2	171	60	85	38	256	98
Probability (3:1)	.7–.8		.3–.5		.7–.8	
Test cross F_1	70	59	36	28	106	87
Probability (1:1)	.1–0.2		.3		.1–.2	

the mutant male-sterile trait was controlled by a single locus with recessive alleles.

Allelic Relationship with *ms₁* and *ms₂* Genes

In the three-way cross (MS Prabhat × F_1 [mutant × ICPL 85010]), involving the *ms₁* gene and the new mutant, all the F_1 plants were fertile, indicating the nonallelic nature of the two male-sterile systems. F_2 progenies of nine three-way cross F_1 plants were studied further to determine the segregation patterns of the two different male-sterile types. Of these, four progenies segregated for fertile and mutant male-sterile types. The pooled segregation within this group across the progenies (Table 2) revealed a good fit to a 3 fertile: 1 sterile ratio ($P < .3$). The remaining five three-way F_2 progenies segregated, besides fertility, for both the translucent-type (*ms₁*) as well as the mutant-type male steriles in a 9:3:4 ratio ($P < .7$). These results suggest that the two male-sterile genes segregated independently and the double recessive resembles the phenotype of a mutant male sterile. The proportion of progenies segregating for only one type of male sterility and those segregating for two types of male sterility fits a 1: 1 ratio ($P < .8$).

In the second three-way cross, where the F_1 (mutant × ICPL 85010) plants were crossed to the arrowhead anther-type male-sterile plants, all the three-way cross F_1 plants were fertile, indicating nonallelic control of these two male-sterility systems. Of the 21 F_2 progenies sown, 9 segregated for fertility and mutant male-sterile types and 12 progenies segregated for fertility and the two male-sterile types. This distribution of the progenies fit to the expected ratio of 1:1 ($P < .7$). In the first group of progenies the pooled segregation for the fertile and mutant sterile plants fit a 3:1 ratio ($P < .2$). In the other group of progenies, the pooled data showed that the segregation for fertile, mutant male sterile, and arrowhead anther-type (*ms₂*) male sterile followed the pattern of a 9:3: 4 ratio with a good fit ($P < .7$). These observations also suggested that the loci for these two male-sterile systems segregated independently and the double-recessive plants resembled arrowhead anther-type male steriles.

The inheritance studies show that the mutant male sterile identified in the population of ICPL 85010 is a new source of male sterility. The expression of this trait is controlled by a single locus with recessive alleles and it is nonallelic to the earlier reported *ms₁* and *ms₂* genes. For this new male-sterile mutant, a gene symbol *ms₃* is designated.

Reddy et al. (1978) reported that in the translucent (*ms₁*) type of male sterile, the anther shape and size develop normally and the male sterility is caused by non-separation of tetrads due to persistent tapetum. In a similar study, Dundas et al. (1981) found that the arrowhead (*ms₂*) type of male sterility is conditioned by the breakdown of microsporogenesis at an early tetrad stage. Therefore, unlike *ms₁*, the shape of anthers in *ms₂* is different and resembles an arrowhead. Saxena et al. (1983), while studying the allelic relationship between *ms₁* and *ms₂* genes, reported that in the double-recessive $ms_1ms_1ms_2ms_2$ genotype of the *ms₂* gene expresses earlier than the *ms₁* gene, and therefore its phenotype resembles that of the *ms₂* male sterile. In the present case, the studies on microsporogenesis of the new mutant were not performed, but the segregation patterns observed in the three-way cross F_2 progenies suggested that in the $ms_1ms_1ms_3ms_3$ genotype, the *ms₃* gene acts earlier than *ms₁*, resulting in a mutant-type phenotype. On the other hand, in the $ms_2ms_2ms_3ms_3$ genotype, the *ms₂* gene is activated earlier than *ms₃* and thus produces a relatively higher frequency of arrowhead-type male steriles (Table 2). Therefore, considering all three genes together, it is postulated that in the ms_2ms_2 genotype the breakdown of microsporogenesis is at the earliest stage, followed by ms_3ms_3 and ms_1ms_1.

This new source of male sterility has enriched the genetic resources of pigeonpea and will help in diversifying the genetic base of male steriles in breeding high-yielding hybrid cultivars and populations. Since it is an inherited trait, it can also be transferred to various agronomically superior backgrounds to develop heterotic hybrids in different phenological groups.

Table 2. Segregation for male sterility in two three-way cross F_1 and F_2 populations

			No. of plants				
Cross	Generation	No. of progeny	Fertile	Mutant sterile	*ms₂* sterile	*ms₁* sterile	Probability
MS Prabhat (*ms₁*) × F_1[a]	F_1	—	23	0	0	0	—
	F_2	4	71[3]	31[1]	0	0	.2–.3
		5	131[9]	51[4]	0	39[3]	.5–.7
	Probability (1:1)	.7–.8	—	—	—	—	—
MS QPL (*ms₂*) × F_1[a]	F_1	—	13	0	0	0	—
	F_2	9	87[3]	38[1]	0	0	.1–.2
		12	85[9]	32[3]	44[4]	0	.5–.7
	Probability (1:1)	.5–.7					

[a] Mutant × ICPL 85010.

[ ] Expected phenotypic proportion.

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Susceptibility to Spontaneous Atherosclerosis in Pigeons: An Autosomal Recessive Trait

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The inheritance pattern for susceptibility to spontaneous (noninduced) aortic atherosclerosis in pigeons was determined by crossbreeding and backcrossing experiments with atherosclerosis-susceptible White Carneau and atherosclerosis-resistant Show Racer breeds. Susceptibility, assessed by the presence of grossly visible lesions at the celiac bifurcation of the aorta at 3 years of age, demonstrated an inheritance pattern consistent with an autosomal recessive Mendelian trait. Cell culture studies indicated that susceptibility is a constitutive property of aortic cells as evidenced by vacuole formation and lipid content in smooth muscle cells from various tissues in susceptible pigeons.

Atherosclerotic cardiovascular disease is the leading cause of death in the United States and other economically developed countries. Genetic factors are currently recognized as major determinants of this pathology (Galton and Ferns 1989), which is considered the most prevalent genetic disorder affecting humans (Funke and Assman 1999). Susceptibility to atherosclerosis is believed to have a complex phenotype probably involving a number of linkages, and this complexity has made attempts to characterize the genetic mechanisms problematic. It is now believed that in addition to numerous genes, significant gene-environment interactions are likely (Breslow 2000). Therefore an understanding of the role of inheritance appears crucial to significantly reducing the death rate.

Since genetic studies of atherosclerosis in humans are difficult, much work has been directed toward animal models, especially the laboratory mouse. The mouse is technically advantageous because of its small size, short generation time, and the availability of many inbred strains (Breslow 2000). However, laboratory mice fed a chow diet do not develop spontaneous atherosclerotic lesions; atherosclerosis must be induced by feeding a diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid. This dietary manipulation presents serious limitations for comparison with humans because of the atypical vascular location of the lesions and their lack of pathologic progression (Smithies and Maeda 1995).

Consequently, from the perspective of pathology, a more relevant animal model than the mouse would be desirable for genetic studies. The White Carneau (WC) pigeon (*Columba livia*) develops spontaneous (naturally occurring) atherosclerosis without elevated plasma cholesterol levels and in the absence of other known risk factors (Clarkson et al. 1959). These noninduced atherosclerotic lesions are morphologically and ultrastructurally similar to those seen in humans (Cooke and Smith 1968; Santerre et al. 1972) and occur at similar geographic sites along the arterial tree (Kjaernes 1981). A variety of studies have clearly demonstrated that susceptibility in the WC resides at the level of the arterial wall (reviewed by St. Clair 1983). Under identical diet and housing conditions, and with similar blood cholesterol levels, the Show Racer (SR) pigeon is resistant to the development of atherosclerosis (Clarkson et al. 1959).

Although atherosclerosis in the pigeon model has been studied for more than 40 years, the inheritance mechanism has not been elucidated. Several early reports (Goodman and Herndon 1963; Herndon et al. 1962; Wagner et al. 1973) suggested a "polygenic mechanism with dominance of factors for resistance" and that factors responsible for lesion initiation were largely independent of factors responsible for lesion progression from initial to advanced stages. Unfortunately the one crossbreeding study reported was confounded by feeding the pigeons a high-fat, high-cholesterol diet to "accelerate" the atherosclerotic pathology, and the individual F_1 and F_2 progeny were not examined (Wagner et al. 1973). The pathologic processes involved in spontaneous atherosclerosis differ dramatically from those of diet-induced or diet-aggravated atherosclerosis in pigeons (Gosselin 1979; St. Clair 1983).

The objective of this study was to define the mechanism for inheritance of susceptibility or resistance to spontaneous atherosclerosis in pigeons by classical breeding studies—that is, crossbreeding and backcrossing. From an examination of the presence or absence of grossly visible, spontaneous celiac lesions at 3 years of age in individual atherosclerosis-susceptible WC, atherosclerosis-resistant SR, and in F_1, F_2, and backcross progeny, we report that susceptibility to spontaneous atherosclerosis is inherited in a pattern consistent with a single-gene, autosomal recessive, Mendelian trait. Smooth muscle cells cultured from several tissues of WC, SR, and F_1 pigeons demonstrate that lipid accumulation in susceptible aorta cells is a constitutive property of WC.

Materials and Methods

White Carneau and Show Racer pigeons were obtained from the University of New Hampshire (UNH) colonies which are housed in fly coops at ambient temperature and allowed free access to water, Purina Pigeon Chow Checkers, and Palmetto Pigeon Health Grit. The colonies were established in 1962 with birds obtained from the Palmetto Pigeon Plant (Sumter, SC) and have been inbred ever since. The colonies are maintained under the supervision of the UNH Animal Care and Use Committee. For crossbreeding, 40 males and females of each breed were paired in 30 inch $\times$ 30 inch $\times$ 30 inch breeding cages containing a roost and two nest boxes. F_1 progeny were removed at 4 weeks of age, banded, placed in a fly coop, and allowed to pair randomly to produce F_2 progeny. Sixteen F_1 males and females were selected and backcrossed with parental WC and SR birds in breeding cages. The backcross progeny were removed at 4 weeks of age, banded, and placed in a fly coop.

At 3 years of age, birds were sacrificed for necropsy. Aortas were removed from the heart to the sciatic trifurcation, opened longitudinally along the dorsal side, and the celiac branch region was dissected free. The most prominent and widely studied spontaneous atherosclerotic lesion in susceptible pigeons occurs at the celiac bifurcation of the aorta and reaches a size that is easily visible on gross examination by 3 years of age (Santerre et al. 1972; Wagner et al. 1973). Lesions were observed as raised, yellow areas under lighted magnification at 1.2$\times$. The location, pathology, and age progression of these lesions have been described in detail by Santerre et al. (1972).

Primary aortic smooth muscle cell cul-