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Influences of Heredity and Environment on Alkaloidal Phenotypes in Solanaceae

MARK J. SOLOMON* and FRANK A. CRANE

Abstract □ Modern biometrical analysis of reported data on *Atropa belladonna* L. indicates varying heritabilities at successive stages of plant ontogeny, exhibiting almost complete additivity at the early flowering stage. A discussion of the importance of this observation with accompanying consideration of more general implications is presented.

Keyphrases □ Phenotypes, alkaloidal, in Solanaceae—heredity, environmental influences □ Solanaceae biovariation—selecting for alkaloidal phenotypes, effects of heredity, environment □ Genetic variation, Solanaceae alkaloidal phenotypes—equations, phenotypic scale diagram

Research efforts involving selection for or against alkaloidal phenotypes are relatively rare in the literature. Such studies are lengthy, involved, and relatively wasteful in time and effort in comparison to the information obtained.

Upon reviewing the literature on Solanaceous alkaloidal biovariation, the authors noted that Sievers (1) had grown plants of *Atropa belladonna* L. for economic reasons during World War I and collected data on total alkaloidal content, expressed as milligrams atropine per gram dry weight of powdered leaf. These data lent themselves to heritability analysis (h^2, the potential for selection under artificial or natural conditions), utilizing modern statistical and biometric techniques (2).[1]

The authors' analysis of Sievers' data is summarized in Table I. Since Sievers analyzed parental types and their respective progeny at different stages of ontogeny, the following conclusions may be drawn:

1. Heritability (h^2) is present.
2. This heritability varies with ontogeny, ranging from high values (maximum value of 1) during vegetative and early flowering stages to lower values in early and late fruiting stages.
3. Heritability is maximum at the flowering stage of ontogeny, reaching almost complete additivity.

To the writers' knowledge, this analysis represents the first application of quantitative genetic techniques to tropane alkaloid biovariation, and it is the first example of quantitative estimation of a relationship of plant ontogenesis to a tropane alkaloid.

[1] The statistical techniques used for this analysis were not known in 1915. It is a tribute to his scientific acumen that Sievers' data could be analyzed over a half-century later to yield these results.

DISCUSSION

Without the information obtained from Sievers' original data (1) and the authors' later interpretation, four models of genetic control appear possible if workers do not postulate genetic and environmental control of alkaloidal phenotypic variation to occur, as most geneticists would assume. These models are:

1. Alkaloidal phenotypes are not inherited; *i.e.*, they are all environmentally controlled.
2. Alkaloidal phenotypes are inherited in Mendelian fashion; *i.e.*, they are monogenic with no environmental control.
3. Alkaloidal phenotypes are the product of multiple allele segregation (with no environmental control).

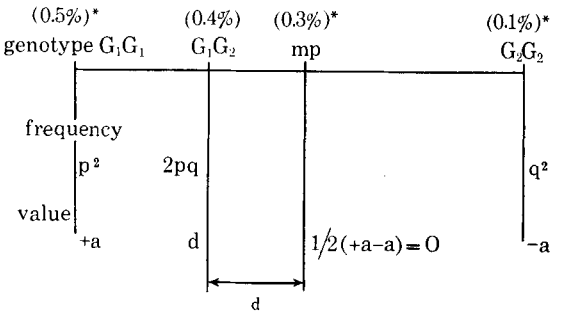


Figure 1—*Alkaloidal phenotypic scale for one gene in the biosynthetic pathway of an alkaloid (26). * A hypothetical case: presence of both dominant alleles would produce a final contribution of 0.5% alkaloidal content. The double recessive G_2G_2 would produce a concentration of 0.1%. If the alleles are additive, the heterozygote A_1A_2 would be 0.3%. If dominance is present, the G_1G_2 genotype would have the value of 0.4%.*

G_1G_1 = *homozygous dominant (maximum contribution to alkaloidal phenotype)*
G_2G_2 = *homozygous recessive (minimum contribution to alkaloidal phenotype)*
G_1G_2 = *heterozygote value (0 point or deviation if dominance is present)*
d = *dominance deviation from midparent*
mp = *midparent value (additive value of G_1G_1 + additive value of G_2G_2 divided by 2)*
α_1 = *average effect when G_1 is substituted for G_2*
α_2 = *average effect when G_2 is substituted for G_1*
α_T = *total average effects on the phenotype*
a = *additive effect due to one allele substitution*
p = *frequency of G_1 allele in population*
q = *frequency of G_2 allele in population*
$p + q$ = *1 since each gene has only two alleles*

Table I—Effect of Ontogenesis on Heritability of Total Alkaloids (Determined as Atropine) in Leaves of *A. belladonna* [Determined from the Data of Sievers (1)]

Statistic	Flowering		Fruiting		Overall
	Before	After	Early	Late	
h^2 [a]	0.8838	0.9520	0.4828	0.3097	0.7240
95% CL_h^2 [b]	0.7874–0.9801	0.8344–1.0000+	0.4007–0.5648	0.2270–0.3924	0.6158–0.8321
Regression equation [c]	$y = 0.1294 + 0.8838x$	$y = 0.0098 + 0.9520x$	$y = 0.2360 + 0.4828x$	$y = 0.4443 + 0.3097x$	$y = 0.2991 + 0.7240x$

[a] h^2 = heritability, s_a^2/s_p^2. When $s_g^2 = s_a^2$, then $h^2 = s_g^2/s_p^2$. [b] 95% CL_h^2 = in 19 of 20 cases the heritability will be between these limits. [c] Regression equation: x = parental value; y = progeny values. Five randomly selected progeny per parent were used to avoid weighting factors. See Falconer (2) for technique, p. 179.

4. Alkaloidal phenotypes are the product of a polygenic system (quantitative inheritance with some environmental control and possible genetic–environmental interaction).

Model 1—In view of Sievers' data and other corroborating evidence, this model is not feasible for *A. belladonna*. Furthermore, other genera in the Solanaceae exhibit evidence of genetic control. The increased numbers of genes in autotetraploids demonstrated by Rowson (3, 4) and recently extended by Solomon and Crane (5) indicate that increased numbers of genes cause increased alkaloid concentration. Stary (6), working with "Poinsettia aneuploids" and "Globe aneuploids," indicated that the 17·18 and 21·22 chromosomes of *Datura* regulate alkaloid production and further implicate genetic control.

The work of Hills *et al.* (7) on crosses of *Duboisia* species and of Evans *et al.* (8, 9) on *Datura* species indicates that genes may regulate the presence or absence of specific alkaloids in interspecific hybrids.

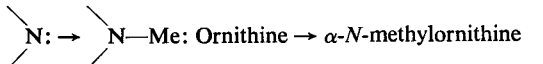
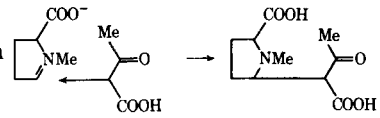
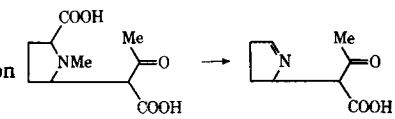
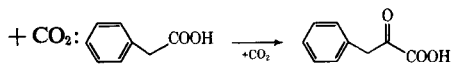
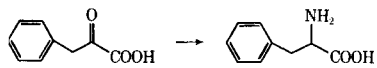
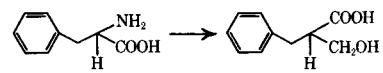
Lecat (10) demonstrated that selection for increased morphine content in *Papaver somniferum* var. *nigrum* is possible. Good chemical screening for alkaloids is based on the empirical premise that if alkaloids are found in a given species, other members of the genus and other genera in the family have a greater probability of producing alkaloids than a random sampling of the plant kingdom. Thus, it appears that genetic control of alkaloidal biovariation and all resulting implications in nautral selection and fitness considerations may be generalized to other families in higher plants.

Model 2—Although Mendelian segregation may be responsible for the presence or absence of specific alkaloids in interspecific genetic studies (7–9) in view of the complex set of biochemical reactions thought to be responsible for various alkaloidal biosyntheses, a single-gene mechanism of inheritance within a particular species should be considered highly unlikely (Table II). Such a mechanism would indicate one gene regulating more than one enzymatically controlled reaction for the biosynthesis of hyoscyamine and possibly even more steps in the biosynthesis of scopolamine. A monogenic mechanism of inheritance would also require that the one gene regulating the alkaloidal phenotype in *Datura* would have the unlikely task of being located on two different chromosomes (the 17·18 and 21·22) at the same time (6).

Model 3—The many types of biochemical transformations involved in the alkaloid biosynthesis already demonstrated for *Datura* effectively eliminate the multiple-allele model as a total explanation of alkaloid inheritance. But even with another model, the possibility of multiple alleles at any genic locus identified with regulating alkaloid inheritance must be considered. This explanation would have to be confined to individual steps in particular biosynthetic schemes rather than to the total biosynthesis of the final alkaloidal product.

Model 4—Quantitative characteristics or metrical characters such as secondary plant product concentrations, *i.e.*, alkaloid concentrations, are usually assumed to be polygenically controlled. Geneticists assume *a priori* that such characteristics have, by their

Table II—Outline of Major Steps Involved in Hyoscyamine and Hyoscine Biosynthesis

Gene [a] (Possible)	Hypothetical Function of Enzyme	Type of Reaction
I	*N*-Methylation	 N: → N—Me: Ornithine → α-*N*-methylornithine
II	Oxidation	Transamination: α-*N*-methylornithine → α-*N* methyl glutamic-γ-aldehyde
III	Ring-closure equilibrium	Ring closure: α-*N*-methylglutamic-γ-aldehyde → *N*-methylpyrrolinium salt
IV	Condensation with acetoacetic acid	Mannich reaction 
V	Oxidation	Oxidative decarboxylation 
VI	Esterification	Tropic acid + tropanone carboxylic acid → hyoscyamine
VII	Carboxylation	 + CO_2:
VIII	Amination	 Transamination:
IX	Intramolecular rearrangement	
X	Oxidation	−2H: Hyoscyamine → 6,7-dehydrohyoscyamine
XI	Oxidation	+O: 6,7-dehydrohyoscyamine → 6-hydroxyhyoscyamine
XII	Oxidation	Oxidation: 6-hydroxyhyoscyamine → hyoscine

[a] Genes are designated by Roman numerals. The dominant alleles would be I_{A1}, I_{A2}, I_{A3} .... The recessive alleles would be I_{a1}, I_{a2}, I_{a3} ....

basic nature, genetic and environmental variation. Since Beadle's (11) statement of the "one-gene–one-enzyme" hypothesis, it has been assumed that one gene regulates one biochemical step through one enzyme. Reexamination of Table II indicates 12 possible biosynthetic reactions resulting from enzymatic activity (12). Thus, there appears to be a high probability that extensive additive genetic variance, estimated by heritability, should be possible. Sievers' data (1) and the writers' analysis indicate that this is the case.

Figure 1 summarizes recognized aspects of established polygenic relationships. A hypothetical numerical example relating to alkaloidal polygenic contributions is given. Significantly, each gene in the biosynthetic pathway has an additive genetic variance which contributes to the rate of selection, either artificial or natural, for or against the final phenotype. This rate of selection, designated as h^2, is the term for heritability. Thus, the presence of heritability in a parent–offspring regression analysis, as demonstrated by Sievers' data, indicates that additive genetic variance is present and that polygenic inheritance applies.

Other types of variation than additive genetic variation are possible. These variances are summarized by the following equation:

$$s_p^2 = s_e^2 + s_s^2 + s_{ge}^2 + s_a^2 + s_d^2 + s_i^2 \qquad \text{(Eq. 1)}$$

$$\text{where } s_a^2 + s_d^2 + s_i^2 = s_g^2$$

and

s_p^2 = total observed variation (phenotypic)
s_e^2 = total environmental variation
s_s^2 = sampling error induced by not using an infinite population
s_{ge}^2 = genetic–environmental variation
s_g^2 = total genetic variation
s_a^2 = total additive genetic variation
s_d^2 = total dominant deviation variation
s_i^2 = total epistatic or intergenic variation

The total average effects of all the genes affecting the final concentration of alkaloids in the phenotype would simulate a normal distribution because of the large number of frequency classes possible. The enzymes listed in Table II need not be specific for a particular step in alkaloid biosynthesis but may be involved in general metabolic activity as well. A polygenic model requires only that a number of enzymes and their respective genes controlling production of the final product, alkaloid, additively produce the final concentration.

Therefore, since the heritability, h^2, varies with ontogeny of the plant in *A. belladonna*, the ratio of the additive genetic variance to the total phenotypic variance also appears to vary. Thus, it appears that the additivity, or the ability to select for or against total alkaloidal variation expressed as milligrams atropine per gram dry weight of powdered leaf, also varies with the ontogeny of the plant.

Most pharmacognosists have stressed the importance of environmental effects on the alkaloids of Solanaceous plants. Such environmental variables as photoperiod, temperature, inorganic nutrition, and their ratios have been reported to influence growth and alkaloid production (13–25). These environmental studies were made on uniform strains of plants, assuming genetic influence as nonvariable. In view of Sievers' data and the authors' analyses, heritability, additivity, and, therefore, the possibility of selection are present. Moreover, the high heritabilities observed in the early vegetative and early flowering phases indicate that natural selection under various environmental circumstances would most probably occur at these stages of ontogeny.

CONCLUSIONS

Modern biometric analysis of reported data on *A. belladonna* indicates that heritability, or the possible rate of selection, either natural or artificial, for the total alkaloids of this species varies with the ontogeny of the plant, reaching maximum levels (almost complete additivity) at the early flowering stage. Reexamination of existing information in pharmacognostical literature indicates that the polygenic model, rather than the three alternative models, appears most satisfactory for explanation of the biovariation of alkaloidal concentrations in specific phenotypes. The presence or absence of particular alkaloids in interspecific hybrids and their genetic segregants may be explained, however, by the usual Mendelian laws.

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