Heterotic Viability in Natural Populations of *Trillium kamtschaticum* Pall¹⁾

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In a series of studies under the general title "Evolution and Variation in *Trillium*", Narise (1956) reported slight but consistent excesses in frequencies of heterozygotes for certain patterns²⁾ of chromosome B in some natural populations of *T. kamtschaticum*. He suggested that superiority of the heterozygotes might be the cause of the observed deviations from the expected frequencies. Since that time a large amount of data concerning chromosome composition of natural populations of this plant has been accumulated (Fukuda and Kozuka, 1958), so that now we can check his suggestion. The purpose of this paper is to present a method for measuring the relative survival values, or viabilities, of genotypes when the only available data are from natural populations.

Re-examination of Breeding system of T. kamtschaticum

Narise (1956) inferred random breeding in most of the natural populations of *T. kamtschaticum* examined by him. Recent analysis with additional data, however, revealed non-random deviations from the Hardy-Weinberg distribution consistently occurring in some of the chromosome pairs in a large number of natural populations of this plant (Kurabayashi, 1961). Usually the deviation was statistically insignificant in each population. This was the reason why Narise disregarded the deviation and concluded random breeding. But now we have sufficient number of populations to show that the deviation is by no means insignificant. Since the expected frequencies of zygotes are greatly influenced by inbreeding, the inbreeding coefficient, f , was computed for each chromosome pair in each population. If there is no differential viability among genotypes, f must be the same for all of the five chromosome pairs in a population. There were, however, clear differences in f among chromosome pairs; generally it was very small, often negative (heterotic), in chromosomes A and B; rather small and rarely negative in chromosomes C and D; and largest in chromosome E. There was little if any sign of heterotic viability in chromosome E. In populations located in Hidaka and Tokachi districts in Hokkaido, Japan, the zygoti-

1) Contribution No. 372 from the National Institute of Genetics, Misima.

2) Patterns of differential staining of chromosome segment under low temperature. For detail see for example Haga and Kurabayashi (1953).

frequencies for chromosome E were very close to what were expected from the Hardy-Weinberg Law. In these populations, therefore, chromosome E may be considered as a relatively neutral chromosome. Hence the f value computed for chromosome E was chosen as an estimate of the inbreeding coefficient for each population.

The f values thus obtained were generally very small in populations in Hidaka and Tokachi districts giving an average of approximately 0.05 (this corresponds to about 10% selfing), but was as high as 0.30–0.70 in the remaining districts. Narise (1956) reported, in one population, *Srr-S*(2) for example, that there was no significant deviation from the Hardy-Weinberg distribution in the zygotic frequencies of chromosome B. When, however, the expected frequencies are re-computed substituting the f value estimated for this population, the deviation now becomes significant; the B. 2–3^h heterozygotes are significantly more frequent than expected. This matter will be discussed in detail elsewhere by others (Kurabayashi, 1961; Fukuda, 1961).

Measurement of relative viability

Let x_{ij} be the adult frequency of the ordered genotype A_iA_j with the fertility w_{ij} ($x_{ij}=x_{ji}$, $w_{ij}=w_{ji}$), and let c_{ij} and t_{ij} be the proportion of survivors of A_iA_j in the next generation and of survivors from seed to adulthood respectively ($c_{ij}=c_{ji}$, $t_{ij}=t_{ji}$). There has been so far few evidences of asexual reproduction (budding) in natural populations of *T. kamtschaticum*, so this will be ignored entirely in the following discussion. There is, however, some evidence for reproduction by budding in an interspecific triploid hybrid, but this will be discussed elsewhere in relation to another subject.

Briefly, the procedure for obtaining the zygotic frequencies in the next generation is summarized as follows. The contribution of gametes from A_iA_j to the next generation is $x_{ij}w_{ij}$. Thus the frequency of A_i in gametes will be,

$$q_i = \sum_j x_{ij}w_{ij}/\bar{w}, \text{ where } \bar{w} = \sum_{i,j} x_{ij}w_{ij}.$$

The proportion of "new adults" of A_iA_j in the next generation (replacement of dead parents) is

$$\{q_i f + (1-f)q_i^2\}t_{ii}/K \text{ if } i=j \text{ and } (1-f)q_i q_j t_{ij}/K \text{ if } i \neq j, \text{ where } K = \sum_i \{q_i f + (1-f)q_i^2\}t_{ii} + \sum_{i,j} (1-f) \times q_i q_j t_{ij}.$$

Since the proportion of A_iA_j survivors in the next generation is $x_{ij}c_{ij}$, we get

$$x'_{ij} = x_{ij}c_{ij} + (1-D)(1-f)q_i q_j t_{ij}/K \quad (i \neq j) \quad \dots \dots (1)$$

$$x'_{ii} = x_{ii}c_{ii} + (1-D)\{q_i f + (1-f)q_i^2\}t_{ii}/K \quad (i = j) \quad \dots \dots (2)$$

where $D = \sum_{i,j} x_{ij}c_{ij}$.

At equilibrium, $x' = x = \hat{x}$ and from equations (1) and (2) we get

$$\hat{x}_{ij}/(1-f)q_i q_j = (1-\hat{D})/\hat{K} \times t_{ij}/(1-c_{ij}) = (1-\hat{D})/\hat{K} \times y_{ij} = \hat{r}_{ij} \quad (i \neq j)$$

$$\hat{x}_{ii}/\hat{q}_i\{f + (1-f)\hat{q}_i\} = (1-\hat{D})/\hat{K} \times t_{ii}/(1-c_{ii}) = (1-\hat{D})/\hat{K} \times y_{ii} = \hat{r}_{ii} \quad (i = j)$$

Note that the left-most expression is the ratio of the observed to the expected zygotic frequency. Since t_{ij} is the proportion of survivors from seed to adulthood

3) Heterozygote for the two patterns of chromosome B; B. 2/B. 3.

(pre-adult viability) and $(1-c_{ij})$ is the proportional loss per generation (adult death rate), y_{ij} measures, or at least reflects the major portion of, the viability of A_iA_j . The frequency of A_iA_j ($=\hat{x}_{ij}$) zygotes is obtained directly from the observed data, but $\hat{q}_i$, the frequency of A_i in gametes, is determined by a set of w_{ij} 's. Since they can not be determined through observations on natural populations, we must assume, for the time being, that all the genotypes have the same fertility. With this assumption, $\hat{q}_i = \sum_j x_{ij}$ and we can then compute $\hat{r}_{ij}$ from the observed data. Since all the $\hat{r}_{ij}$'s are equal to y_{ij} multiplied by the same quantity $(1-\hat{D})/\hat{K}$, we may measure y_{ij} relative to a certain constant multiplied by $(1-\hat{D})/\hat{K}$. One example would be the unweighted average of y_{ij} ($=\bar{y}_{ij}$) in a population.

$$R_{ij} = y_{ij}/\bar{y}_{ij}$$

It is easily shown that, when there is no difference in viability among genotypes, $\hat{r}_{ij} = 1$. Therefore any deviations in $\hat{r}_{ij}$ from unity must be the indication of differential viability of genotypes, although the magnitude might be influenced somewhat by fertility differences. Thus the value of R_{ij} actually reflects both the differential fertility and viability.

Generally the number of plants sampled per population is small. Therefore if there are many chromosome patterns with low frequencies it is convenient that one choose the "most interesting" two patterns and group the remaining patterns into one class.

Applications and Discussions

Since an extensive discussion will be given by Kurabayashi (1961), only one group of populations from Saruru is considered in this report. The Saruru area is located close to Cape Erimo in Hokkaido, at the boundary of Hidaka and Tokachi districts. In this area three populations, *Srr-S*(2), *Srr-S*(3) and *Srr-N*, have been investigated. There, so far, only two patterns with approximately the same frequency have been found in chromosome B, while more than twenty have been found in chromosome A. The frequency of each chromosome pattern in the three populations is given in Table 1 and zygotic frequency in Table 2.

There are many patterns in chromosome A but among them two, A. 5 and A.

Table 1. Frequencies of patterns found in chromosomes A and B in three populations in the Saruru area

Chromosome pattern and frequency (%)											
Population	A. 5	30	2	33	36	17	Others	No. of plants	B. 2	3	No. of plants
<i>Srr-S</i> (2)	27	22	13	8	7	3	20	45	54	46	45
<i>Srr-S</i> (3)	19	24	11	4	4	6	32	40	56	44	40
<i>Srr-N</i>	37	19	6	7	6	4	21	45	58	42	45
<i>Mean</i>	27	22	10	6	5	5	25		56	44	

Table 2. Frequencies of chromosome combinations for A and B observed in three populations in the Saruru area

Chromosome combination and its observed frequency											
Population	A. 5-5	5-30	30-30	5- <i>x</i> *	30- <i>x</i>	<i>x</i> - <i>x</i>	Total	B. 2-2	2-3	3-3	Total
<i>Srr-S</i> (2)	0	8	2	16	8	11	45	12	25	8	45
<i>Srr-S</i> (3)	0	5	2	10	10	13	40	10	25	5	40
<i>Srr-N</i>	5	10	1	13	5	11	45	10	32	3	45

* All patterns except 5 and 30 were grouped into a class *x*.

30, appear to be the most frequent patterns in this area and may deserve special attention. Therefore, all patterns except these two were grouped into one (class *x*). The numerical values of R_{ij} were then computed *assuming that each population is in a state of equilibrium*. These values are presented in Table 3.

Table 3. R_{ij} values of chromosome combinations for A and B in three populations in the Saruru area

Chromosome combination and its R_{ij} value									
Population	A. 5-5	5-30	30-30	5- <i>x</i>	30- <i>x</i>	<i>x</i> - <i>x</i>	B. 2-2	2-3	3-3
<i>Srr-S</i> (2)	0.00	1.98	0.52	1.71	1.03	0.79	0.78	1.49	0.73
<i>Srr-S</i> (3)	0.00	1.58	0.99	1.30	1.04	1.11	0.86	1.40	0.71
<i>Srr-N</i>	0.90	2.24	0.24	1.27	0.60	0.72	0.62	2.08	0.32
<i>Mean</i>	0.30	1.93	0.58	1.43	0.89	0.87	0.75	1.66	0.59

Although there are some population to population differences the order of magnitude is fairly constant.

It is very interesting to note that the heterozygote of the two high frequency patterns in chromosome A, A. 5-30, has the highest viability although their homozygotes have the lowest values. The value of R_{xx} is larger than that of either homozygotes, A. 5-5 or A. 30-30, but the class *x* contains many patterns and most of the plants in this class are heterozygous.

Thus superior viability of heterozygotes appears to be a general phenomenon in natural populations of *T. kamtschaticum* and if two (or more) patterns with especially high viability as their heterozygotes are introduced into a population or population group, they will be accumulated to form the characteristic chromosome patterns of a given locality even if their homozygotes have low viabilities.

The fitness of a genotype is the resultant of many components, which interrelate in an exceedingly complicated way in the course of development. Therefore the present analysis serves only as a preliminary survey. However, the following points are worth mentioning. The weighted average viability of a given chromosome pattern in

a population, say A_i ($= Y_i$), is computed to be

$$Y_i = \sum_j x_{ij} R_{ij} / q_i$$

The numerical values of Y_i and its weighted average, $\bar{Y}$, are presented in Table 4.

Table 4. Y_i values of various A and B chromosomes in three populations in the Saruru area

Population	Y_i value of the indicated chromosomes						
	A. 5	30	x	$\bar{Y}$	B. 2	3	$\bar{Y}$
<i>Srr-S</i> (2)	1.78	1.34	1.15	1.36	1.15	1.18	1.16
<i>Srr-S</i> (3)	1.37	1.16	1.15	1.19	1.17	1.20	1.18
<i>Srr-N</i>	1.44	1.52	0.89	1.26	1.51	1.81	1.66
<i>Mean</i>	1.53	1.34	1.06	1.27	1.28	1.40	1.33

In chromosome B, there is practically no difference between Y_3 and Y_2 in *Srr-S*(2) and *Srr-S*(3), whereas a fairly big difference is seen in the *Srr-N* population. Since $Y_2 < \bar{Y}$ the frequency of B. 2 in this population must decrease, or the assumption of a state of equilibrium for *Srr-N* was wrong (note that the frequency of B. 2 is the highest in *Srr-N*). Probably *Srr-S*(2) and *Srr-S*(3) populations are close to equilibrium where we expect $Y_2 = Y_3 = \bar{Y}$.

In chromosome A, Y_x is smaller than $\bar{Y}$ in all the three populations, hence this class will be eliminated, and at the final equilibrium the whole population, at least a large portion of it, will be occupied by the two patterns A. 5 and A. 30. Thus equilibrium has not yet been reached for chromosome A. In *Srr-S*(2) and *Srr-S*(3) populations Y_5 is larger than Y_{30} suggesting an increase of A. 5 in its frequency relative to A. 30, while in *Srr-N*, Y_{30} is slightly larger than Y_5 . As shown in Table 1 the frequency of A. 5 relative to A. 30 is much smaller in the former two populations than the latter. Keeping all of these facts in mind the equilibrium frequencies of A. 5 and A. 30 are expected to be about 0.60 and 0.40 respectively. Note that this final state in chromosome A is very close to what was already established in chromosome B.

From these computations it is suggested that chromosome B in the Saruru area might be the one which has succeeded in producing two "specific" patterns while chromosome A may be now in the process of establishing a similar state.

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Summary

Viability, one of the major components of fitness, has been studied in natural populations of *T. kamtschaticum*. The main points discussed are as follows.

1. The f value computed for chromosome E was chosen as an estimate of in-breeding coefficient for each population. This was on the assumption that this chromosome was relatively neutral in viability.

2. With this f value, a method was proposed to measure the viabilities of genotypes when the only available data are from natural populations.

3. The method was applied to actual data obtained from natural populations of *T. kamtschaticum* and it was concluded that the heterotic viability is a general phenomenon in natural populations of this plant.

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