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Molecular Heterosis at the Conalbumin Locus in the Ring-Necked Pheasant *(Phasianus colchicus)*

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Summary. - Egg whites of *Phasianus colchicus* containing conalbumin of the heterozygous type show a stronger inhibitory effect on the growth of *Saccharomyces cerevisiae* than egg whites with conalbumin of the common homozygous type. This fact is interpreted as an example of heterosis at the molecular level.

Although the usual explanation for the maintenance for biochemical polymorphism is heterozygote superiority, experimental proof of such a mechanism is scarce. In fact, the sole phenomenon in which the heterozygotes enjoy a physiological benefit is the human hemoglobin S case (Allison 1955, 1964): the HbA/HbS individuals appear to be protected from malaria. The biochemical and physiological reasons for the better resistance to infection by *Plasmodium falciparum* are, however, complex, and still poorly elucidated (Power 1975).

Recently, Frelinger (1971, 1972, 1973) and Frelinger and Crow (1973) demonstrated that the heterozygous form of egg-white conalbumin of *Columba livia* inhibits the growth of *Saccharomyces cerevisiae* more strongly than does the common form. This simple mechanism was postulated to explain the maintenance of conalbumin polymorphism. In the present study we suggest that a similar conclusion can be brought forward in the case of *Phasianus colchicus*.

Materials and Methods

74 eggs laid by different females were obtained from controlled lines of the Office National de la Chasse. The analytical technique used was horizontal electrophoresis in starch gel, in tris-citrate buffer, pH 8.6; proteins were stained by amido black. Conalbumin is easily identified directly on the slide by its pink colour. Total proteins were measured by the method of Lowry *et al.* (1951). Conalbumin content of egg-white was estimated from optical density curves measured with a densitometer on polyacrylamide gels. The electrophoresis in polyacrylamide was performed using tris-glycine buffer, pH 8.3. The wild strain of *Saccharomyces cerevisiae* used was grown on synthetic medium YNB 21, both kindly supplied by Dr. Y. Surdin-Kerjan. Growth was estimated by following the curve of optical density over eighty hours. The experiment was performed in tubes each containing 10 ml of the medium and 25 µl of egg-white; the initial amount of yeast added corresponded to an O.D. of 50. The measure of binding of Fe was adapted from the method of Ramsay (1957a, b).

Results

Figure 1 shows a diagram of electrophoretic separation of egg-white proteins on starch gel (Lucotte and Kaminski 1976), completed following Baker *et al.* (1966). In the sample studied, conalbumin formed either a slow band (phenotype S) or two bands (phenotype FS).

The total amount of protein was 140.4 mg/ml, conalbumin representing an average of 16%. Such a high amount was considered to be characteristic of Gallinaceae (Feeney and Komatsu 1966). The densitometric evaluation of the amount of conalbumin showed that adding up the values corresponding to the two bands of phenotype FS equals the amount corresponding to the single band of phenotype S.

Table 1 indicates the proportions of different phenotypes observed in the studied sample. These proportions are compatible with the hypothesis of panmictic equilibrium, but they differ notably from those reported in various populations of *Columbia livia* (Frelinger 1972) in which the two alleles had nearly equal frequencies.

Figure 2 shows the curves of growth of *Saccharomyces cerevisiae* on 3 egg-white samples chosen from each of the two phenotypes. In spite of considerable individual variability, it appears that the growth of *Saccharomyces cerevisiae* is more strongly inhibited by conalbumin in the heterozygous than in the homozygous state. The observed differential inhibition is demonstrated by different levels of growth rather than by different rates at which these levels are reached.

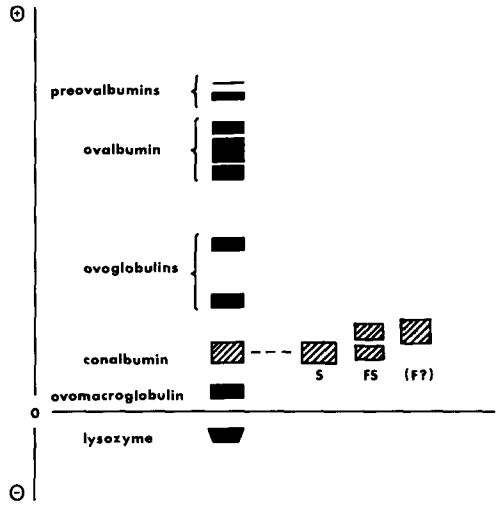

Fig. 1. Schematic electropherogram in starch gel of egg-white proteins of *Phasianus colchicus*; variability at the level of conalbumin showing three phenotypes; the phenotype F was not encountered in the population sample studied

Table 1. Proportions of phenotypes of conalbumin. Numbers in brackets are those expected if the panmictic equilibrium were realized. N: number of egg whites

N	S	FS	F
74	67(66,1)	7(6,3)	0(0,1)

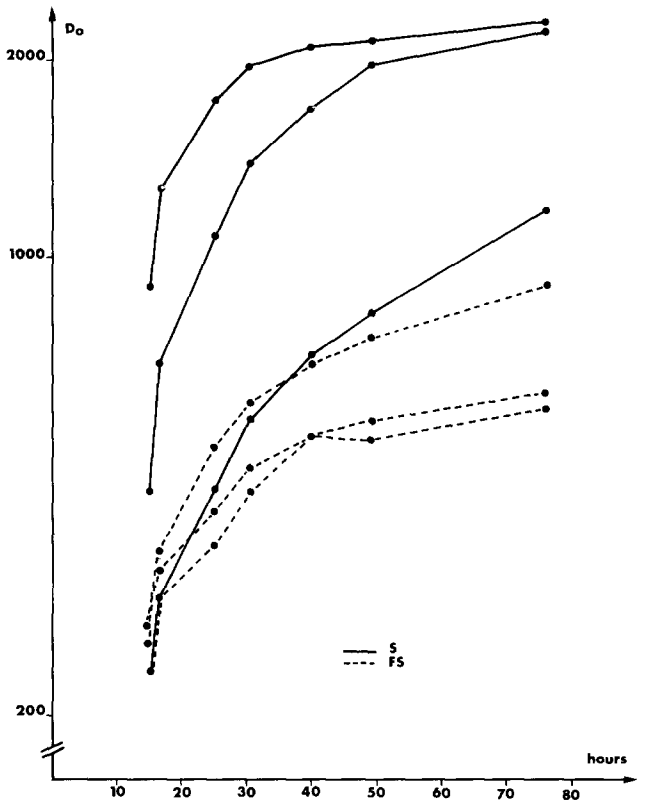

Fig. 2. Growth of *Saccharomyces cerevisiae* in the presence of two types of conalbumin

The iron-binding capacity of the two phenotypes of conalbumin, measured on 3 samples of egg-white bearing each form, was estimated as FS/S = 1,27.

Discussion

In addition to its role in iron transport, conalbumin appears to be the major agent for the chemical protection of the egg against microbial infection (Board and Fuller 1974). Conalbumin acts by chelating iron and thus inhibiting microbial growth (Feeney and Nagy 1952; Garibaldi 1960; Garibaldi and Bayne 1960, 1962). The use of *Saccharomyces cerevisiae* as a model for the study of growth inhibition is justified by the effective parasitism of eggs by yeasts in natural conditions, as well as by other iron-dependent microorganisms such as *Candida albicans*, *Shigella dysenteria*, *Mycobacterium tuberculosis*, *Salmonella typhimurium* and *Pasturella septica*.

On the other hand, both conalbumin and its plasma homologue, transferrin, are polymorphic in numerous breeds of domestic flock and other avian species, such as *Streptopelia tranquebarica*, *Anser anser*, *Cairina moschata*, *Larus atricilla*, *Pica pica*, *Corvus cornix*, *Larus ridibundus*, *Melanitta nigra*, *Columba palumbus*, *Streptopelia risoria*, *Alectoris graeca*, *Francolinus erckeli* (in Wright 1974), *Alectoris rufa* and *Colinus virginianus* (Lucotte and Kaminski 1976).

To check the hypothesis of Frelinger, we chose a species of Gallinaceae, characterized by a high level of conalbumin. Even though the observed proportions of phenotypes at that locus do not correspond to equal gene frequencies, the egg-white showing the heterozygote phenotype of conalbumin inhibits the growth of *Saccharomyces cerevisiae* more strongly than the common type. This stronger inhibition leads to a lower level of the stationary phase of growth. Such a phenomenon reveals a case of molecular heterosis: the eggs containing the heterozygous form of conalbumin gain an advantage over the common form in the face of infection.

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