SEASONAL VARIATION IN *PGM* HETEROZYGOSITY IN HONEYBEES (*APIS MELLIFERA* L.)

N. HADIMOGULLARI, M. KENCE, A. KENCE

Department of Biology, Middle East Technical University, 06531 Ankara, TURKEY
Tel.: +90 312 2105176, fax: +90 312 2101289, E-mail: aykut@metu.edu.tr

Introduction

Research on allozymes have revealed enormous genetic variability and indicated a positive association between heterozygosity and fitness. There has been numerous studies showing association between enzyme heterozygosity and fitness components such as viability, developmental stability, growth rate and fecundity in various plant animal species. These parameters are also associated with metabolism as well environmental conditions (MITTON, 1997).

During a study to determine the genetic variation in southeastern Anatolian honeybee populations we have observed very high heterozygosity in Pgm locus. When we attempted to reproduce the results in order to search for the cause of this high level of heterozygosity, we could not succed in our first attempt. Therefore we have taken several samples at different times and we have noticed that there is a seasonal change in heterozygosity level at Pgm locus. Here, we aim to report the seasonal change that occur in the heterozygosity level of Pgm enzyme.

Materials and Methods

A total of 1010 individuals from 201 colonies were sampled from 85 locations of seven provinces in southeastern Anatolia in the period of 1988-2000. Some provinces were visited both in summer and winter seasons while others were sampled in summer season only.

Approximately 3000 worker bees were collected and they were put in small plastic bottles, labeled and fed with Turkish Delight (water+saccha-rose+starch) and brought alive to the laboratory. Honeybees were dissected, thoraces of honeybees were ground and homogenates were kept frozen until needed for electrophoresis.

Four enzyme systems (Esterase, 3.1.1.1; Hexokinase, 2.7.1.1.; Malate dehydrogenase, 1.1.1.40; Phosphoglucomutase, 2.7.5.1) known to be polymorphic in *Apis mellifera* were utilized as biochemical markers. Starch-gel electrophoresis, gel and sample preparation and experimental conditions have been reported previously (KANDEMIR et al., 2000). Gene frequencies, enzyme heterozygosities, and population heterozygosities were calculated according to NEI (1995) using BIOSYS (Swofford and SELANDER, 1981). Goodness of fit of genotype frequencies to HARDY-WEINBERG expectations was tested by χ^2 – test (SOKAL and ROHLF, 1995).

Results and Discussion

Two of those four isozyme loci, Pgm and Hk were polymorphic, Mdh, Est-3, Pgi, and Me were found to be monomorphic. Pgm was the most polymorphic locus with two alleles and it exhibited polymorphism in all of the provinces. The frequencies of the most common alleles for Pgm and Hk loci and χ^2 values for deviation of genotypic frequencies are given in Table I. Only Hatay and Gaziantep samples deviate from H-W equilibrium, in other provinces genotypic frequencies for both isozymes are in agreement with H-W equilibrium. Only in Hatay and Gaziantep we have sampled both in winter and summer, whereas in other provinces we have sampled only in summer. Pgm was different from other enzyme systems in that heterozygosity level was seen to change seasonally. It exbitited high level of heterozygosity in the samples collected in winter. On the other hand the genotype frequencies of those collected in summer were in Hardy-Weinberg equilibrium. The honeybees collected in winter were compared to those collected in summer for the deviation of genotypic frequencies from Hardy-Weinberg equilibrium and the results of the Chi-square test are given in Table II. The difference between the samples collected in winter and summer are apparent in Pgm, while in Hk locus there is no significant difference between the winter and summer sample frequencies.

Allele frequencies for the most common allele of the Pgm and Hk loci in winter and summer samples and mean heterozygosities are given in Table III. The allelic frequencies for Pgm differ again between the winter and summer samples, while for Hk locus there are no differences between the winter and summer samples. This have not been reported in literature previously.

Table I

The most common allele frequencies of Pgm and Hk isoenzymes and Chi-square values for the deviation of genotype frequencies from the Hardy-Weinberg equilibrium

Provinces	Pgm-75 freq.	Pgm		Hk-100 freq.	Hk	
		χ^2 – value	P		χ^2 – value	P
Hatay	(172) 0.81	8.826	0.003	(81) 0.99	0.000	1.000
Gaziantep	(239) 0.71	35.199	0.001	(169) 1.00	0.000	1.000
Adiyaman	(98) 0.93	0.454	0.500	(50) 0.92	0.328	0.567
Malatya	(91) 0.96	0.124	0.725	(102) 0.99	0.015	1.000
Mardin	(57) 0.90	0.718	0.397	(71) 1.00	0.000	1.000
Diyarbakir	(272) 0.99	0.011	0.916	(57) 1.00	0.000	1.000
Sanliurfa	(81) 0.91	0.669	0.413	(133) 0.99	0.004	0.951

Table II

Chi-square test for the deviation of genotypic frequencies from H-W equilibrium in different seasons

Populations	Pgm			Hk		
	χ^2 – value	df	P	χ^2 – value	df	P
Winter	390.740	1	0.001	0.062	3	0.996
Summer	0.003	1	0.959	0.000	1	1.000

Table III

The frequency of the most common alleles of the Pgm and Hk *loci* winter and summer samples

Populations	Pgm-75	Hk-100	Direct	H-W exp.
Winter	0.552	0.980	0.496±0.456	0.269±0.230
Summer	0.995	0.996	0.009±0.002	0.009±0.002

Seasonal changes in the level of heterozygosity was not limited to Hatay and Gaziantep. We have sampled honeybees all over Turkey between 1994 and 1997 KANDEMIR et al., 2000). In that study we have found significant deviations in favor of heterozygotes in some of the locations. The locations that have shown the most significant deviations were sampled at the beginning of April. Also in Elazig and Artvin honeybee populations sampled in winter we have observed 100% heterozygosity.

There are large amount of studies on the impact of heterozygosity on the individual's as well as population's fitness. Even a single locus can have major impact on the whole animal physiology. GOULSON (1993) observed that the genotype at the locus Pgm affected the length of time for which individual butterflies (*Maniola jurtina*) could fly continuously. The author found that in temperatures of 29^{o}C the individual butterflies homozygous for Pgm-100 can continuously fly for longer intervals than individuals of other genotypes. And he concluded that during cold conditions heterozygotes may fly longer than individuals homozygous for Pgm-100.

CORNUET et al. (1995) in their study on thermostability of Mdh allozymes of honeybees found that the allozyme coded for by MM genotype was unstable under heat and it was inactive after treatment of heating at 65^{o}C for three minutes. This property of M allele was also observed in heterozygotes (FM and SM) where it gave rise to reduction in activity of the heterodimer enzymes. Those heterodimers were less active than FS, FF, and SS which were not affected by the rise in temperature. MESSIER and MITTON (1996) studied Mdh polymorphism in *Apis mellifera* and found that heterozygotes had lower levels of fluctuating asymmetry than did homozygotes. HARRISON et al. (1996) in their study suggested that endothermic insects including honeybee, *A. mellifera* have the ability to regulate their thoracic temperature according to the air temperature. As air temperature increased from 20^{o}C to 40^{o}C the metabolic heat production and wingbeat frequency during hovering, agitated or loaded flight decreased by 40% (HARRISON et al., 1996). This may be actively controlled by a homeostatic mechanism which may include oxygen consumption or energy production. Honeybees can maintain the power to fly and to remain active outside the hives across a wide range of air temperatures by the features that enables them to thermoregulate their thorax (ROBERTS and HARRISON, 1999). The variation in Pgm locus of honeybees may be the part of homeostatic mechanism that controls the thermoregulation of their thorax.

A study of variation in ten polymorphic enzyme loci of fire ant colonies of northern Georgia, USA, showed that all but one locus, Pgm, to be in Hardy-Weinberg equilibrium (ROSS, 1992). In polygynous populations Pgm-3AA queens are killed by the workers around the time oogenesis is initiated but Pgm-3Aa and Pgm-3aa queens are not, because Pgm-3AA queens are more efficient than other genotypes in reproductive development (ROBINSON et al., 1997). More fecund queens are identified by the help of pheromones and are killed by worker ants (FLETCHER and BLUM, 1981).

A study on the relationship between Mdh polymorphism and developmental stability in honeybee showed that the Mdh heterozygotes had lower levels of fluctuating asimmetry than homozygotes (MEISSER and MITTON, 1996). A study by WATT and his colleagues on Pgi polymorphism in Colias butterflies has shown that heterozygotes had superior survival, their males had higher mating success compared to homozygote males (WATT, 1977; WATT et al., 1985).

There are studies also on individual heterozygosity concerning a number of loci. For instance American oysters (*Crassostrea virginica*) which were homozygous for a number of loci including Pgm, consumed more oxygen as did heterozygous ones under both normal and stress conditions (KOEHN and SHUMWAY, 1982). An association between enzyme heterozygosity and growth rate was also reported for *C. virginica* that the growth rate increased with heterozygosity at several loci (SIGNH and ZOUROS, 1978; ZOUROS et al., 1980). Another study on common killifish (*Fundulus hetroclitus*) with Pgm and Est loci revealed that the double heterozygote individuals had the highest fitness; the individuals that were heterozygous for Pgm exhibited higher viability than homozygous ones (MITTON and KOEHN, 1975).

In a recent study by VERRELLI and EANES (2001) reported that there is extensive geographic variation of protein polymorphisms at the Pgm locus in *Drosophila melanogaster* and allozyme alleles show latitudinal clines and the best explanation for the pattern of clinal variation at the Pgm locus is one that includes selection.

Pgm is an important enzyme is glycolytic pathway which regulates the glucose metabolism. There may be a relationship between the increase in heterozygosity level and survival. Individuals heterozygous for Pgm may utilise glucose more efficiently than homozygotes. So they may consume less honey compared to homozygotes which is very important for survival in winter. This may cause the homozygotes to be selected against at the beginning of the winter season. Various hypotheses explaining the seasonal variation in the level of heterozygosity will be tested in order to understand the reason of this interesting phenomenon in honeybees.

REFERENCES

Cornuet J.-M., Oldroyd B.P., Crozier R.H., Unequal thermostability of allelllic forms of Malate dehydrogenase in honeybees. *Journal of Apicultural Research* 34 (1) (1995), 45-47

Fletcher D.J.C., Blum M.S., Pheromonal control of dealation and oogenesis in virgin queen fire ants. *Science* 212 (1981), 73-75

Goulson D., Allozyme variation in the butterfly, *Maniola jurtina* (Lepidoptera: Satyrine L.). Evidence for selection. *Heredity* 71 (1993), 386-393

Mitton J.F., Selection in natural populations. Oxford University Press Inc., 1997

Harrison J.F., Fewel H.F., Roberts P.R., Hall H.G., Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* 274 (1996), 88-90

Kandemir I., Kence M., Kence A., Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations of Turkey. *Apidologie*, 31 (2000), 343-356

Koehn R.K., Shumway S.E., A genetic/physiological explanation for differential growth rate among individuals of the American oyster, *Crassoditrea virginica* (Gmelin). *Mar. Biol. Ltrs* 3 (1982), 35-42

Mitton J.B., Koehn R.K., Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus*. *Genetics* 79 (1975), 97-111

Messier S., Mitton J.B., Heterozygosity at the malate dehydrogenase locus and developmental homeostasis in *Apis mellifera*. *Heredity* 76 (1996), 616-622

Nei M., Molecular Population Genetics and Evolution. North-Holland, Amsterdam, 1975

Roberts S.P., Harrison J.F., Mechanisms of thermal stability during flight in the honeybee *Apis mellifera*. *The Journal of Experimental Biology* 202 (1999), 1523-1533

Robinson G.E., Fahrbach S.E., Winsto M.L., Insect societies and the molecular biology of social behavior. Review article. *BioEssays* 19 (1997), 1099-1108

Ross K.G., Strong selection on a gene that influences reproductive competition in a social insect. *Nature* 355 (1992), 347-349

Singh S.M., Zouros E., Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution* 32 (1978), 342-353

Sokal R.R., Rohlf F.C., Biometry. Third Ed. W.H. Freeman and Company, NY, 1995

Swofford D.L., Selander R.B., BIOSYS-1: A computer program for the analysis of allelic variation in genetics. Release 1., 1981

Verrelli B.C., Eanes W.F., Clinal variation for amino acid polymorphisms at the Pgm locus in *Drosophila melanogaster*. *Genetics* 157 (2001), 1649-1663

Watt W.B., Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: Biochemical and population aspects. *Genetics* 87 (1977), 177-184

Watt W.B., Carter P.A., Blower S.M., Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of *Colias* butterflies. *Genetics* 109 (1985), 157-175

Zouros E., Singh S.M., Miles H.E., Growth rate in oysters: An overdominant phenotype and possible explanations. *Evolution* 34 (1980), 856-867