A NEW PHASE BEGINS FOR THE USDA-ARS RUSSIAN HONEY BEE BREEDING PROGRAM

by THOMAS E. RINDERER[a], LILIA DE GUZMAN[a], and ROBERT DANKA[a]

Ten years ago, the staff of the USDA, Honey Bee Breeding, Genetics and Physiology Laboratory in Baton Rouge, Louisiana visited the Primorsky Territory on the Pacific coast of Russia in an effort to find honey bees resistant to *Varroa destructor*. The area was chosen since it has had one of the longest known associations of *Apis mellifera* and *V. destructor*. In the mid 1800s, settlers from European Russia brought the western honey bee to the area[1] which had a native population of the eastern honey bee, *A. cerana*, and its external mite parasite, *V. destructor*[2]. This importation of *A. mellifera* occurred decades before varroa was scientifically described[3]. It also happened during the time when moveable frame beekeeping equipment was just beginning to be adapted throughout the world. We speculated that these conditions might have resulted in selection for resistance to *V. destructor* in the population of *A. mellifera* which was descended from the early importations.

Work with these bees has included preliminary evaluations of honey bee colonies in far-eastern Russia[4], detailed study of colonies in Russia, importation of Russian queens through quarantine into the United States[5, 6], a detailed comparison of the response to varroa mite infestations of Russian and Italian colonies[7], additional studies of selection for resistance to varroa mites[7, 8, 9, 10, 11] resistance to tracheal mites[12, 13, 14] and studies of honey production[15, 16]. Much of this work reports the results of field trials of groups of daughter queens from the most promising of the imported queen lines and the incorporation of these lines into a closed population breeding program. Reports also include research to identify advantageous management tools that complement and enhance the mite resistance of the imported stock[17, 18, 19, 20]. These efforts have been ongoing for over 10 years (Table 1).

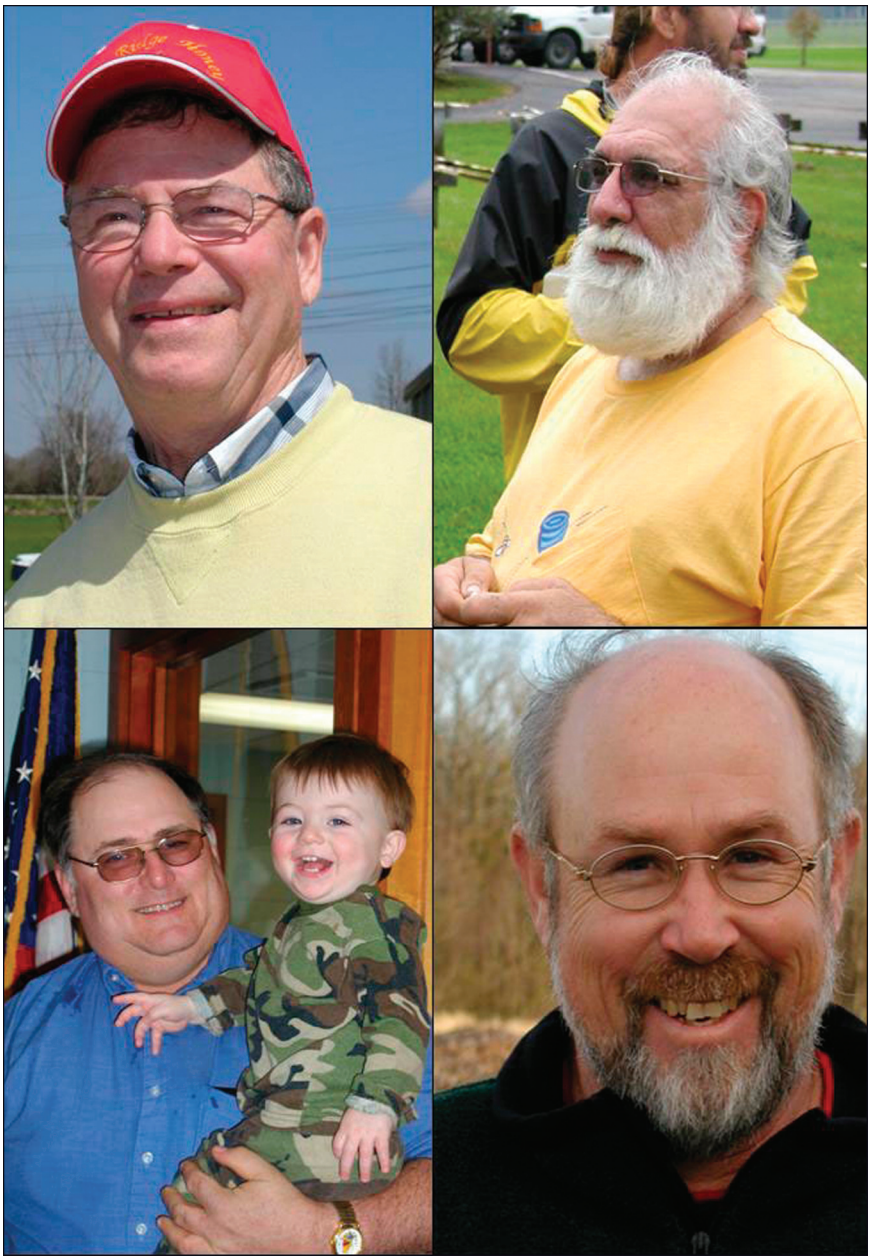

Figure 1. Beekeepers cooperating with the breeding project. (l-r) Manley Bigalk (Iowa), Charlie Harper, Louisiana (Cooperative Research and Development Agreement partner), Hubert Tubbs (Mississippi), and Steve Bernard (Louisiana).

[a] USDA-ARS Honey-Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820-5502 (e-mail: Trinderer@ars.usda.gov, Ldeguzman@ars.usda.gov, RDanka@ars.usda.gov)

We imported five groups of queens from 1997 to 2002 from Russia. In total, 362 queens were imported through a USDA-APHIS-approved quarantine station. Colonies from individual queens were evaluated and the best of them were used as mothers of groups of sister queens which were established in colonies in Mississippi, Iowa and Louisiana. These colonies were commercially owned and managed by cooperating beekeepers (Steve Bernard and Charlie Harper in LA, Hubert Tubbs in MS, Manley Bigalk, in IA (Fig. 1). The cooperation of these beekeepers has been and continues to be essential to the program. Lines which showed a combination of resistance to varroa mites, tracheal mites and good honey production were retained for a breeding program. Lines were evaluated for resistance to varroa in all locations and resistance to tracheal mites in field colonies in Iowa and by a short test in Baton Rouge. After the selection of breeder lines, they were assigned to one of three groups, which are used to make natural matings, according to a plan designed to avoid inbreeding and maintain genetic diversity, while still allowing for continued selection for mite resistance, honey production and winter hardiness.

Year	Country	Activity
1994	Russia	·Initial examination of Russian honey bees ·Establishment of five-year cooperative research agreement with Russian Academy of Sciences
1995	Russia	·Collection of Russian queens from varied sources ·Russian cooperators begin resistance test with collected queens
	USA	·Begin similar test with domestic stock in Baton Rouge, LA
1996	Russia	·Finish test of collected Russian queens
	USA	·Finish test of domestic stock in Baton Rouge, LA
1997	Russia	·Collected 100 Russian queens from varied sources in Russia for shipment to the United States
	USA	·Quarantine imported Russian queens
1998	Russia	·Russian cooperators begin screening queens
	USA	·Finish quarantine procedures for 1997 imported queens ·Evaluate colonies of each imported queen, choosing the best for further testing ·Begin comparative test of Russian and domestic colonies
1999	Russia	·Establish test colonies with newly collected queens ·Ship the most varroa resistant queens from the 1998 test to the United States
	USA	·Finish comparative test of Russian and domestic colonies ·Begin multi-state sibling tests ·Begin breeding and propagation with three queen lines, one in each breeding block ·Evaluate colonies of each imported queen, choosing the best for further testing ·Quarantine Russian queens imported in 1999
2000	Russia	·Establish test colonies with newly collected queens ·Ship the most varroa resistant queens from the 1999 test to the United States
	USA	·Release first selected stock to the beekeeping industry ·Begin new multi-state sibling tests ·Begin breeding and propagation with three queen lines, one in each breeding block ·Evaluate colonies of imported queens, choosing the best for further testing ·Quarantine Russian queens imported in 2000 ·Finish quarantine procedures for 1999 imported queens
2001	Russia	·Establish test colonies with newly collected queens ·Ship the most varroa resistant queens from the 2000 test to the United States
	USA	·Second release of selected stock to the beekeeping industry ·Begin new multi-state sibling test ·Continue breeding and propagation with selected lines ·Evaluate colonies of imported queens, choosing the best for further testing ·Quarantine Russian queens imported in 2001 ·Finish quarantine procedures for 2000 imported queens
2002	Russia	·Ship most varroa resistant queens from the 2001 test to the United States ·End of 5-year cooperative research agreement with Russian Academy of Sciences
	USA	·Third release of selected stock to the beekeeping industry ·Begin new multi-state sibling test ·Continue breeding and propagation with selected lines ·Evaluate colonies of imported queens, choosing the best for further testing ·Quarantine Russian queens imported in 2002 ·Finish quarantine procedures for 2001 imported queens
2003	USA	·Fourth release of selected stock to the beekeeping industry ·Begin new multi-state sibling tests ·Continue breeding and propagation with selected lines ·Evaluate colonies of imported queens, choosing the best for further testing ·Finish quarantine procedures for 2002 imported queens
2004	USA	·Fourth release of selected stock to the beekeeping industry ·Begin new multi-state sibling tests with the last of the imported queens ·Continue breeding and propagation with selected lines
2005	USA	·Fourth release of selected stock to the beekeeping industry ·Begin more intensive selection within selected queen lines in cooperation with beekeepers

Table 1. Time Line of the Russian Honey Bee Breeding Program

In 2004, we concluded multi-state field trials of the last of the imported queen lines. The results were similar to prior years; some lines were retained for the breeding program and other lines, because of either below average honey production or poor resistance to parasitic mites, were culled. The average varroa mite population growth of the Italian control colonies was similar to the theoretical calculation (Fig. 2), and the average honey production of the Italian colonies was near the apiary average (Fig. 3). The average colony of the Russian standard line had a small mite population growth (Fig. 2) and good honey production (Fig. 3). Of the lines being tested for inclusion in the breeding program, colonies of the tan and white lines had large mite population growths (Fig. 2) and the colonies of the tan line had honey production that was consistently below apiary averages (Fig. 3). Because of these poor performances, the tan and white lines were discarded and not considered for use in the program. The green, orange, purple, red, yellow and blue lines were included in the program for further improvement.

Over the course of the importation and testing of imported portions of the program, 362 queens were imported and their colonies were individually evaluated. The best 42 queens were used to produce daughters for the multi-state field tests. Eighteen queen lines have been retained from the original 362 queens imported and have been organized into three breeding groups (Table 2). We have changed the marking system used to identify individual queen lines. There are a limited number of colors that are useful for helping to find marked queens. Hence, different groups had lines that had the same mark, creating confusion for many people. Only by knowing both the color mark and the year a specific queen line was released is it possible to know the line of the queen. Now that we have chosen all of the breeder lines, we are able to assign each line a unique and permanent color marking scheme. To do this and still use colors, which are easy for most people to distinguish, we have given all but one line a two-color- mark designation. The one exception is the line which in prior years has served as a "Russian standard" in our field evaluations. This line is

Breeding Block	Block Color	Line Color	Year Selected From Sibling Test
A	White	Dark Blue	1999
		Green	2004
		Purple	1999
		Red	2004
		White	2004
		Yellow/ Dark Blue	1999
B	Light Blue	Dark Blue	2000
		Orange	2003
		Red	2003
		Tan	2003
		White	2004
		Yellow	2004
C	Neon Yellow	Dark Blue	2004
		Green	2001
		Orange	2001
		Purple	2001
		White	2001
		Yellow	2001

Table 2. Russian honey bee breeder lines, their assigned breeding block, color designation and the year they were selected for the program.

Group	2005	2006	2007	2008
A	Test	*Release*	Test	Test
B	Test	Test	*Release*	Test
C	*Release*	Test	Test	*Release*

Table 3. The schedule for the testing and release of the three-groups of Russian queen lines.

unique since it has undergone more intensive selection than the other lines and has a three-color designation, white/yellow/blue.

The emphasis of the program has now shifted from identifying breeder lines to intensifying selection within breeder lines. Each year, daughter queens of the lines which are candidates for release as breeder lines two years later will be used to requeen entire apiaries. Two to four apiaries will serve test sites for each of the lines. To achieve this schedule, in 2005 we will release selected breeder lines of block C, conduct the last of a single year test on block A and begin a two- year test on block B. In 2006, we will release breeder queens from some lines of block A, continue the progeny test of block B and begin a progeny test of block C queen lines. In 2007, we will release selected breeder queens from some lines of block B, continue the test of block C and begin a two-year test of Block A. Thus, by the end of three years, all of the queen lines will be in one or another stage of a two-year evaluation in two or more apiaries (Table 3).

Once queens have been introduced into colonies, baseline estimations of the number of varroa and tracheal mites in the colonies will be done. Then, the colonies will be completely managed by the cooperating beekeeper who will make records for each colony concerning honey production, temper and overwintering. Colonies will not be treated for mites as long as they are candidates for inclusion into the breeding program. Colonies that require treatment before final evaluations will not be used for breeding. Final evaluations will start with the records produced by cooperating beekeepers. Those colonies ranking the highest will then once again be examined for beekeeping traits such as colony size, brood nest quality, nest organization, temper, the presence of disease, and the presence of the original queen. We will then estimate the number of varroa to calculate the mite population growth in the colonies that are still candidates for selection. Promising colonies will then be evaluated for resistance to tracheal mites. The queens in the selected colonies will be used to

Fig. 2

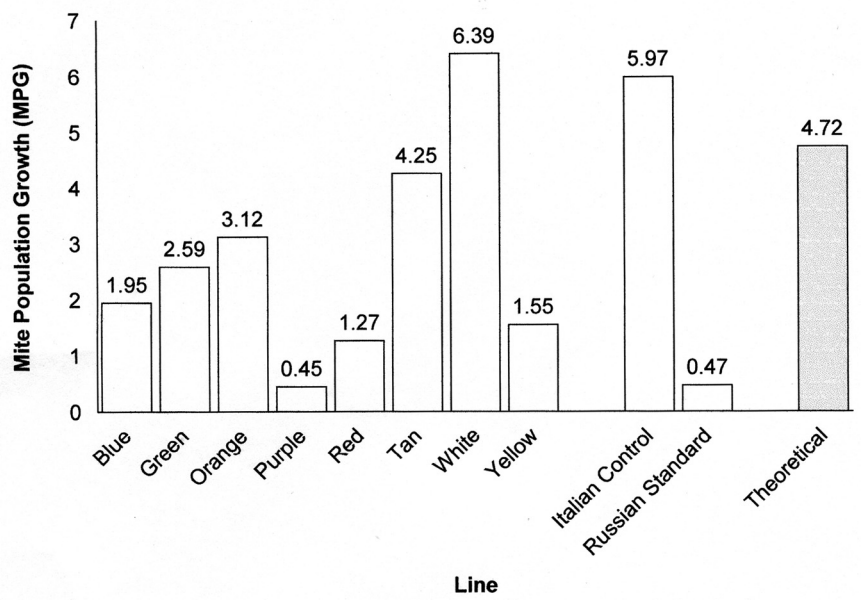

Figure 2. Average mite population growth (MPG) expressed as fold increase in *V. destructor* populations in colonies of nine Russian queen lines and Italian control colonies for the 2004 multi-state trial.

Fig. 3.

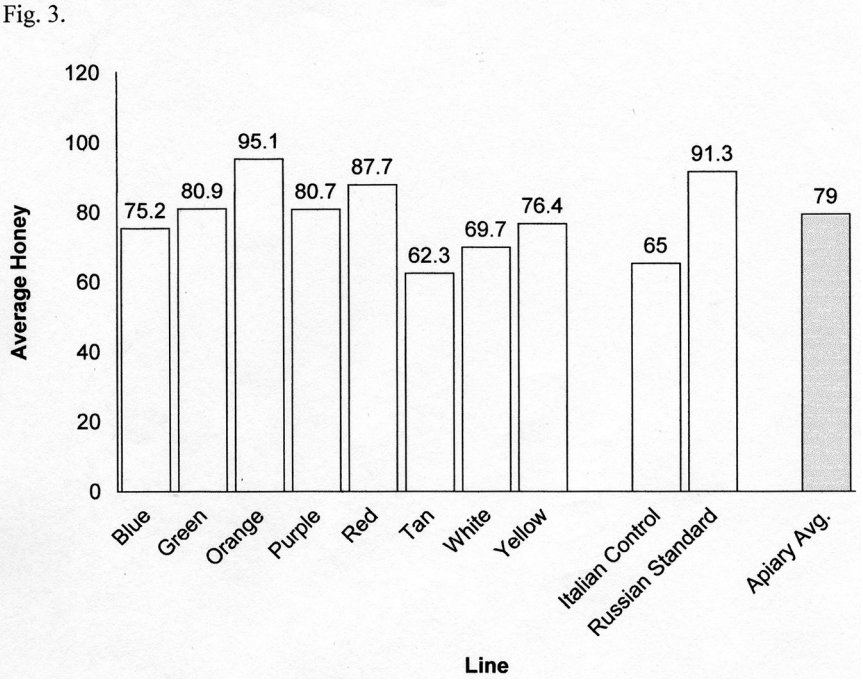

Figure 3. Average honey production of colonies of nine Russian queen lines and Italian control colonies for the 2004 multi-state trial.

propagate the next generation, which will be further selected and may be used for release to the beekeeping industry.

Cooperating beekeepers will play an even more important role than they have in the past. They will collect most of the information that will be used to evaluate potential breeder queens. Staff of the Baton Rouge bee laboratory will make evaluations of the comparative mite resistance of candidate breeder colonies and work with the cooperating beekeepers in the selection of lines to release and breeder colonies to continue the selection in each line. Hence, the program is not and will not be a static program. Each year the lines will be undergoing a selection process involving a reasonably large number of daughters of the selected breeder colonies. Hopefully this process will permit the continued improvement of the lines' mite resistance and honey production. All three of our long-standing cooperators will be involved in this process of line selection. Mr. Harper will continue to be the holder of the Cooperative Research and Development Agreement and the source of breeder queens for the industry. Mr. Tubbs and Mr. Bigalk will continue to get queens of the various lines in order to evaluate them as possible breeders.

This new phase of the Russian honey bee program is more heavily dependent upon beekeepers. This shift will continue as the program goes through the next few years. The program has three important goals. First, we will further improve the stock and make it more desirable to more beekeepers. Second, the stock selection and maintenance program will be conducted by beekeepers with ARS staff serving as advisors. Third, ARS staff will shift their activities to other breeding and genetic goals. We will be working to develop marker-assisted breeding using DNA technology that can then be used to still further increase the varroa and tracheal mite resistance and other valuable traits of Russian and other valuable stocks of honey bees. We will determine if there is a relationship between resistance to varroa mites and resistance to the small hive beetle. We will also continue to develop management techniques that allow beekeepers to take full advantage of the Russian and other genetically improved stocks for honey production and supplying pollination services.

References

1. **Crane, E. 1978.** Beekeeping round the world. *Bee World.* 59:164-167.
2. **Kuznetsonv, V. N. and Proshchalykin M. Yu.** Chinese wax bee *Apis cerana cerana* F. (Hymenoptera. Apidae) in the south of the Russian far east. 56pp. Russian Academy of Sciences. Vladivostok.
3. **Oudemans, A. C. 1904.** Acarological Note VIII. On a new genus and species of parasitic mite. *Notes Leyden Museum* 24 (July):216.
4. **Danka, R. G., Rinderer, T. E., Kuznetsov, V., and Delatte, G. T. 1995.** A USDA-ARS Project to evaluate resistance to *Varroa jacobsoni* by honey bees of far-eastern Russia. *Am.. Bee J.* 135: 746-748.
5. **Harris, J. Rinderer, T. E., Kuznetsov, V., and Delatte, G. T. 1995.** A USDA-ARS Project to evaluate resistance to *Varroa jacobsoni* by honey bees of far-eastern Russia. *Amer. Bee J.* 135:746-748.
6. **Rinderer, T. E., Kuznetsov, V. N., Danka R. G., and Delatte, G. T. 1997.** An importation of potentially varroa-resistant honey bees from far-eastern Russia. *Amer. Bee J.* 137: 787-789.
7. **Rinderer, T. E., de Guzman, L. I., Delatte, G. T., Stelzer, J. A., Lancaster, V. A., Kuznetsov, V., Beaman, L., Watts, R., and Harris, J. W. 2001.** Resistance to the parasitic mite *Varroa jacobsoni* in honey bees from far-eastern Russia. *Apidologie* 32: 381-394.
8. **De Guzman, L. I., Rinderer , T. E., Delatte G. T., Stelzer J. A., Beaman L. D., Harper C. 2002.** Hygienic behavior by honey bees from far-eastern Russia. *Amer. Bee J.* 142: 58-60.
9. **Harris, J. and Rinderer, T.E. 2004.** Varroa resistance in hybrid ARS Russian honey bees. *Amer. Bee J.* 144:797-799.
10. **Rinderer, T. E., Delatte, G. T., de Guzman, L.I., Williams, J., Stelzer, J. A. and Kuznetsov, V. 1999.** Evaluations of the Varroa-resistance of Honey Bees Imported from Far-Eastern Russia. *Amer. Bee J.* 139: 4, 287-290.
11. **Rinderer, T. E., de Guzman, L. I., Delatte, G. T., Stelzer, J. A., Williams, J. L., Beaman L. D., Kuznetsov, V., Bigalk, M., Bernard S. J. and H. Tubbs. 2001.** Multi-state field trials of Russian honey bees 1. Responses to *Varroa destructor* 1999, 2000. *Amer. Bee J.* 141:658-661.
12. **De Guzman, L. I., Rinderer, T. E., Delatte, G. T. Stelzer, J. A., Beaman, G. and Kuznetsov, V. 2002.** Resistance to *Acarapis woodi* by honey bees from far-Eastern Russia. *Apidologie* 33: 411-415.
13. **De Guzman, L. I., Rinderer, T. E., Delatte, G. T., Stelzer, J. A., Williams J. L., Beaman, L.D., Kuznetsov, V., Bernard, S. J., Bigalk, M. and Tubbs. H. 2001.** Multi-state field trials of ARS Russian honey bees: 3. Response to *Acarapis woodi*, 1999, 2000. *Amer. Bee J.* 141: 810-812.
14. **De Guzman L. I., Rinderer T. E., Delatte G. T., Stelzer J. A., Beaman L. D. and Harper C. 2001.** An evaluation of far-eastern Russian honey bees and other methods for the control of tracheal mites. *Amer. Bee J.* 141: 737-741.
15. **Rinderer, T. R., de Guzman, L. I.,. Delatte, G. T, Stelzer, J. A., Lancaster, V. A., Williams, J. L., Beaman, L. D., Kuznetsov,V. Bigalk, M., Bernard, S. J. and Tubbs, H. 2001.** Multi-State Field Trials of Russian Honey Bees: 2. Honey Production 1999, 2001. *Amer. Bee J.* **141: 726-729.**
16. **Rinderer, T.E., de Guzman, L.I., Delatte, G.T. and Harper C. 2003.** An evaluation of ARS Russian honey bees in combination with other methods for the control of Varroa mites. *Amer. Bee J.* 143, 410-413.
17. **Rinderer , T. E., de Guzman, L. I., Harris, J., Kuznetsov, V., Delatte, G. T., Stelzer, J. A., & Beaman,L. 2000.** The Release of Russian honey bees. *Amer. Bee J.* 140: 305-307.
18. **Rinderer, T. E., de Guzman, L. and Harper, C. 2004.** The effects of co-mingled Russian and Italian honey bee stocks and sunny or shaded apiaries on varroa mite infestion level, worker bee population and honey production. *Amer. Bee J.* 144:481-485.
19. **Tubbs. H., Harper, C., Bigalk, M., Bernard, S.J., Delatte, G. T., Sylvester, H. A. and Rinderer, T. E. 2003.** Commercial management of ARS Russian honey bees. *Amer. Bee J.* 144. 819-820.
20. **Cargel, R. A. and Rinderer, T. E.** Unusual queen cell construction and destruction in *Apis mellifera* from far-eastern Russia. *J. Apic. Res.* In press.