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BeeConSel - Joint Effort for Honey Bee Conservation and Selection

# DELIVERABLE 7

# Modelling of countries' specific population structure under mating control options

BeeConSel is funded by Iceland, Liechtenstein and Norway through the EEA and Norway Grants Fund for Regional Cooperation.

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Deliverable driver: S Andonov

Authors: B Dahle, M Kovačić, J Prešern, A Uzunov

Photos: M Kovačić, Š Kodre



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# EXECUTIVE SUMMARY

One of the BeeConSel goals was designed to test and to provide tailor-made solutions for breeding programs in beneficiary countries. Mating control in honey bees is needed for breeding purposes, where the next generation originates from the most desirable queens. Breeding programs are designed to ensure genetic gain for the wide population, where usually not all stakeholders are participating with their own stock. In such a program, it is essential that participants will agree on the minimum criteria and methods used. But, to ensure the sustainability of the breeding program many other aspects have to be closely monitored, such as genetic diversity, inbreeding, and genetic gain, but also to be cost-effective.

Within the BeeConSel project, beneficiary countries gained information about the the perfomance of tested methods (see D6), some of which were shown as promising through the paternity assignment to the known drone producing colonies. A questionnaire regarding specific needs was prepared and distributed among partners. Collected answers, outputs of WP1 (queen production costs when using a specific type of mating control) and the information on performance of tested methods were used as an input to modelling and for the optimization of the breeding programs for three main aspects: maximising genetic gain, maintaining genetic variation, and minimising the cost of mating control. The process of modelling involved several steps. Initially, the performance of each single mating control method was evaluated, followed by the optimization of simulatd breeding program for each of the beneficiary countries for specific population structure, the success and cost of mating control methods in use, maximizing genetic gain and minimizing the inbreeding and costs. The modelling was performed for a single trait for ten non-overlapping generations.

In Croatia, two mating control methods were modelled, geographical isolation in deep forest and biological saturation with drones in flat lands. The balanced modelling assumed the use of both methods in parallel with adjustments in selected queens. The model showed that in 10 generations inbreeding could be kept under 4 %, genetic variation maintained, and genetic gain could be 1.4 units. Although the balanced approach resulted in 13 % lower genetic gain it can ensure a long-lasting breeding program and sustainable genetic gain. For the needs of the breeding program in Macedonia, initially, four mating control methods were assumed, but in the final modelling, only two (highland Isolated locations and instrumental insemination) were considered to be used simultaneously. The outcome of the modelling for ten generations was that the inbreeding could be kept under 3 %, genetic variation maintained, and a relative genetic gain of 1.9 units. Reducing genetic gain for 0.7 units in comparison with pure instrumental insemination was high, but one should also consider the capacity constraints of instrumental insemination and the price of a mated queen. In Slovenia two methods of mating controls were considered, alpine high-altitude valleys and instrumental insemination. When both methods were combined the genetic gain in 10 generations could reach 2.1 units, while keeping the genetic variation similar to the initial level, and the inbreeding under 4 %.

Genetic improvement is slow and cumulative. With the modelling, the likely outcomes of different mating control schemes were projected and proved that the breeding programs are feasible with remarkable genetic gain. It should be noted that environmental and management effects were neglected since they are hardly predictable for the next 20 years. Also, for simplicity, the modelling included only one moderately inheritable trait, which *per se* is not a completely realistic approach in the breeding program. A deeper analysis of the capacities, structure, costs, and approaches is suggested before decisions making.



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# BACKGROUND

Within the BeeConSel Project, several specific mating control methods in honey bees were tested, as described in detail in previous deliverables. Some of them showed promising performance as confirmed by the determination of patrilines. Geographically isolated places were very successful in alpine valleys and island while proving modestly successful in deep forest isolation and highlands micro-locations. Biological saturation tested in flatlands also demonstrated promising use in controlled mating. The alternative approach of time isolation (moonlight method) had modest success, which varied among seasons and modalities. However, another option of controlled mating is the use of instrumental insemination, a method that can be seamlessly incorporated into routine practices when performed by well-trained and skilled operators. The beneficiary countries tested the approaches identified as the most promising to be implemented in the breeding programs and are best adapted to the availability of resources and capacities. In the breeding programs, it is crucial that next generation originates from the most desirable animals, e.g., to reproduce individuals with the highest genetic value. The genetic value of the queens and drones can be for production (honey yield), behaviour (hygienic, defensive, swarming), vitality (colony strength, brood development, resilience to parasites and diseases), maintaining diversity (breed against inbreeding, tolerable effective population size, keeping alleles sufficient of CSD - Complementary Sex Determiner on the population).

The general idea for the implementation of controlled mating was to obtain protection of the local honey bee population through continuous improvement. The breeding program should thus ensure sustainable use of the population by exploring genetic gain, which keeps the focus/interest of the local beekeepers. Hence, within the WP4 of the BeeConSel project, the second aim was to design a tailor-made breeding model for the national-specific breeding program.

To understand how the breeding program can be designed in beneficiary countries, a specific questionnaire was prepared and distributed among partners. Collected answers and data collected in WP1 (queen production costs when using specific type of mating control) were used to optimise the breeding program for three main aspects: maximising genetic gain, maintaining genetic variation, and minimising the cost of mating control. In addition, information about population size, colonies for drones and virgin queen production, colonies in performance testing and targeted annual queens' production were obtained for each country. All the data were combined with the success of different mating control approaches, their optimal frequency in use and estimated costs. For the purpose of understanding the performance and consequences, the first step was to model the performance of the breeding program with a single mating control method. This initial analysis was then followed by the balanced approach where the genetic gain was predicted, combining optimal contribution selection, and accounting for the cost of each controlled mating system. The trait considered in the modelling process had an initial mean value of 0 and deviation of 1, where genetic variation was set to 0.3 and residual to 0.7. No environmental effect was assigned. The modelling was done for each mating approach as a separate case for 10 non-overlapping generations and the values presented here are the average of 20 iterations. From colonies, the newborn queens were produced in 1st year, and drones in 2nd year. The colonies were selected on their phenotypic level, while the mating was positive assortative. After simulating 10 generations, the variance components and breeding values were estimated.

The population modelling was done in SIMplyBee (Obšteter et al., 2022), evaluation in BLUPF90 (Misztal et al., 1999), and optimisation in R (RStudio team, 2020).

# MODELLING SPECIFIC BREEDING PROGRAMS

## Croatia

### Inputs in modelling process

The total population size of honey bees in HR is about 460000 colonies kept by about 9000 beekeepers. Herewith, a case study was built with a single breeding program where geographical isolation in deep forest and biological saturation with drones in flat land fashion were established as mating control methods. In this simulation, we set the number of mated queens to 15000, which will be used for own replacement of the queens by breeders and the need of the beekeepers outside the breeding program. The breeding population was set to be within 5 apiaries with a total of 1500 colonies. The performance testing was modelled to be done on 250 colonies. 400 colonies were assigned for queen production, while a number of Drone Producing

Colonies (DPCs) was set to 100 + 300 in reserve. The ancestor's information for the dam side would be available for 1500 colonies, while about 500 queens would have full pedigree, e. g. both parents known. For the initial phases of the breeding program, there was no plan for genotyping. More details regarding the background of the breeding program are given in Table 1.

Table 1. Input data for the breeding program modelling

| **Parameter** | **Number** |
|---|---|
| Number of honey bee colonies: | 1500 |
| Number of beekeepers | 5 |
| Number of colonies under performance testing | 250 |
| Number of beekeepers using performance testing | 5 |
| Number of annually genotyped queens | 0 |
| Annual queen production | 15000 |
| Colonies used for queen production | 400 |
| Number of DPC | 400 |
| **Known ancestors of queens for queen and drone production** | |
| Only dam | 1500 |
| Only sire | |
| Both | 500 |
| Beekeepers in use of mating control | 5 |
| **Mating control in use** | |
| **Deep forest Isolation** | |
| Cycles | 2 |
| Virgin queens per cycle | 30 |
| DPC | 20 |
| Patriline success | 65 % |
| Losses | 25 % |
| **Flatland saturation** | |
| Cycles | 6 |
| Virgin queens | 450 |
| DPC | 150 |
| Patriline success | 85 % |
| Losses | 25 % |
| **Estimated cost rate** | |
| Mainland Isolated | 5 |
| Mainland saturation | 1 |



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## Mating control implications

Based on D6, we assumed that 65 % of the mating of the queen will be performed by drones from controlled DPCs in deep forest situation. Two cycles with 30 queens and 25 % losses on site have been foreseen. The relative cost of a mated queen on this mating station would be 5. The modelled genetic progress was run for 10 generations. All the virgin queens and DPCs originated from the rest of the population. Due to the presence of foreign drones and high diversity within DPCs, the estimated inbreeding at generations 10 would be practically only 1-2 % compared to the first generation. The genetic variance would be maintained. The genetic gain is expected to be about 0.97 units (Figure 1).

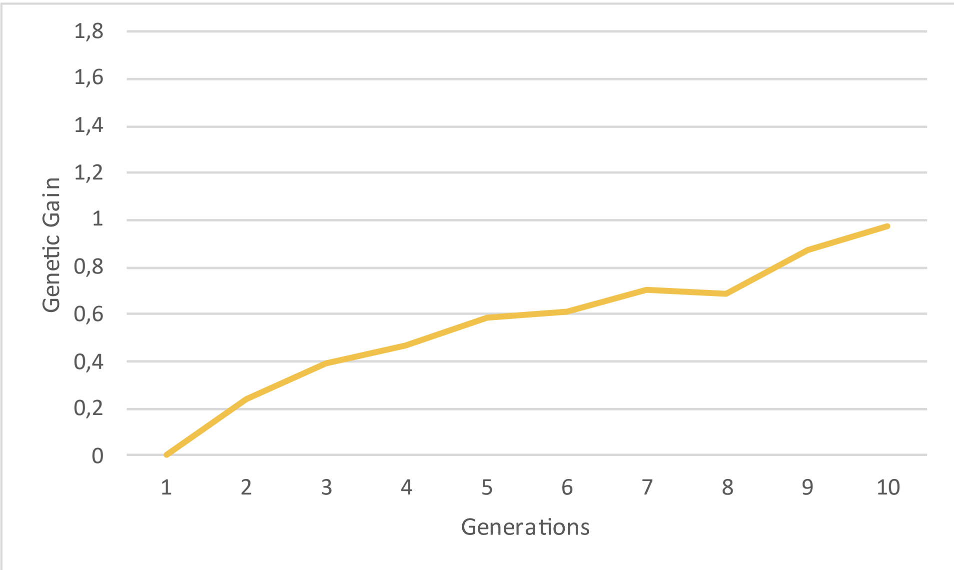


Figure 1. Genetic gain for 10 generations on mating station isolated by deep forest

The simulation of biological saturation of the mating sites with many drones was set to provide the contribution of an average of 85 % (D6) known origin drones to the mated queen. Also, the system can work fine if many virgin queens need to be mated in several cycles on the same spot. On the other hand, yearly introduction of many queens mated with the same DPCs every year can narrow the population's genetic composition. In the modelling, we set mating of 450 queens per cycle and at least 6 cycles were used; the number of DPCs was set to about 150. Such a structure would lead to a


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certain genetic progress of 1.7 units as shown in Figure 2. Using biological saturation as the only mating control method in the breeding program, the inbreeding could raise up to 11 % in the 10th generation, and up to 20 % depletion of initial genetic variation should be expected.

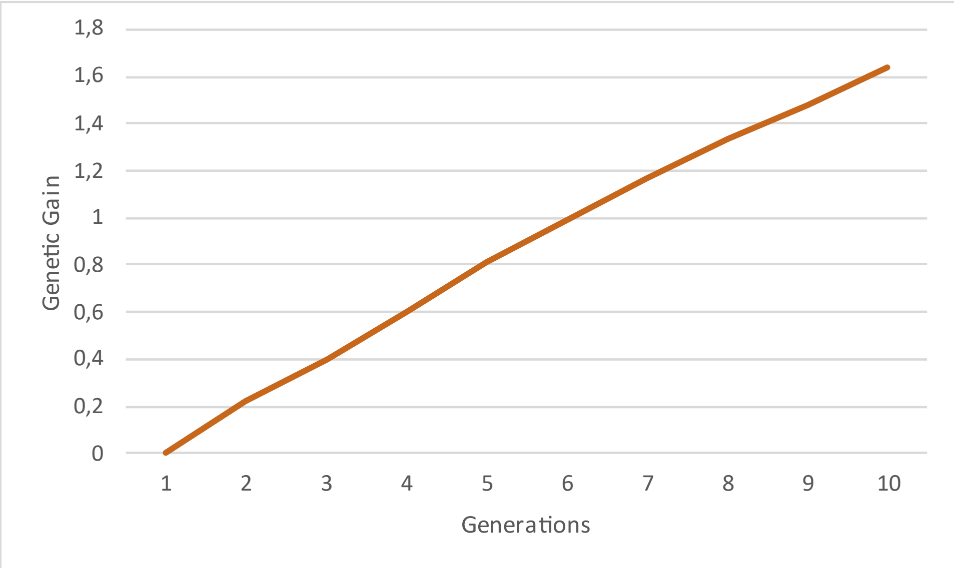


Figure 2.Genetic gain for 10 generations on mating station of biological saturation with drones

## Genetically balanced output

Balanced genetic gain could be obtained with both mating control methods used in parallel with adjustments in selected queens. Such model shows that in 10 generations inbreeding would be kept under 4 %, genetic variation maintained, and relative genetic gain of 1.4 units. However, the price of the genetically balanced approach comes with the 13 % lower genetic gain and an increased relative cost per produced queen of 2.3 %. Nonetheless, it can ensure a long-lasting breeding program and sustainable genetic gain for a long period.



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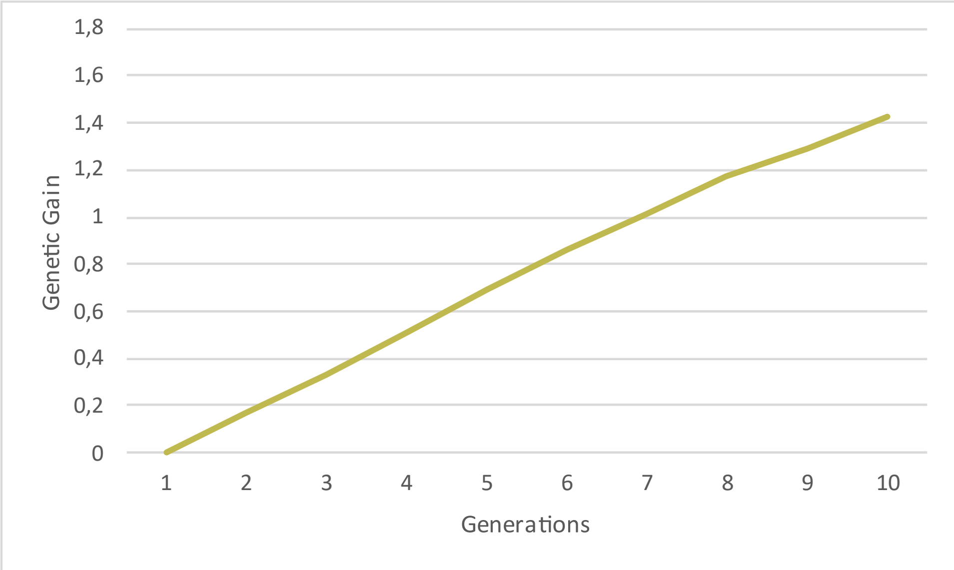


Figure 3. Genetic gain for 10 generations balanced for the inbreeding and genetic variation

# N. Macedonia

## Inputs in modelling process

The total population size of honey bees in N. Macedonia is about 240000 colonies kept by about 6000 beekeepers (Dimitrov et al., 2021). As before, several breeding programs could theoretically operate in the country, and only a minor part of the beekeepers would participate in each breeding program. Herewith, a case study was built on single breeding program where geographical isolation was considered in two highlands sites, and one location on an island. Additionally, the breeding program would utilize instrumental insemination. The annual production of mated queens was set to 2000, used for own replacement of the queens by the breeders. The breeding population would be within 20 apiaries with total 600 colonies, where the performance testing would be done. For the production of next generation of queens, 100 colonies would be selected, while production of Drone Producing Colonies (DPCs) was set to 80. The ancestor’s information for the dam side would be available for 600 colonies, while 20 queens would have full pedigree, e.g. both parental sides are known. No genotyping was considered for the breeding program. More background details for the breeding program are presented in Table 2. The modelled breeding program


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engaged mating control on two highland isolated locations, as it was shown in D5 and D6 (Toranica and Nikiforovo), one island (Golem Grad), and instrumental insemination. The two highland isolated locations were foreseen similar in reliability (one had 5 % higher than the other), and the cost of performing mating control in each location was the same. For simplicity, in the modelling it was considered as one where a higher reliability of 65 % was considered. After considering the cost of using the mating station on the island, where 85 % reliability was confirmed, it turned out that it is not an option for routine use in the breeding program since the higher reliability for lower cost can be obtained through instrumental insemination.

Table 2. Input data for the breeding program modelling

| **Parameter** | **Number** |
|---|---|
| Number of honey bee colonies: | 10000 |
| Number of beekeepers | 20 |
| Number of colonies under performance testing | 600 |
| Number of beekeepers use performance testing | 20 |
| Number of annually genotyped queens | 0 |
| Annual queen production | 2000 |
| Colonies used for queen production | 100 |
| Number of DPC | 80 |
| **Known ancestors of queens for queen and drone production** | |
| Only dam, | 600 |
| Only sire | 20 |
| Both | 20 |
| Beekeepers will use mating control | 20 |
| **Mating control in use** | |
| **Higland Isolated locations 1** | |
| Cycles | 3 |
| Virgin queens per cycle | 600 |
| DPC | 40 |
| Patriline success | 65 % |
| Losses | 20 % |
| **Highlands Isolated locations 2** | |
| Cycles | 3 |
| Virgin queens per cycle | 600 |
| DPC | 40 |
| Patriline success | 60 % |

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| Parameter | Number |
|---|---|
| Losses | 20 % |
| **Island Isolation** | |
| Cycles | 2 |
| Virgin queens per cycle | 100 |
| DPC | 25 |
| Patriline success | 85 % |
| Losses | 30 % |
| **Instrumental insemination** | |
| Cycles | 1 |
| Virgin queens per cycle | 200 |
| DPC | 20 |
| Patriline success | 100 % |
| Losses | 20 % |
| Estimated cost rate | |
| Mainland Isolated | 1 |
| Island Isolation | 10 |
| Instrumental insemination | 8 |

## Mating control implications

Based on D6 results, we assumed that 65 % of the matings at the mating station were performed by drones from DPCs at highland Isolated sites. Also, we have assumed 3 cycles with 600 queens, and 20 % losses on site. The relative cost of mated queen on this mating station was set to be 1. The genetic progress was simulated for 10 generations. All the virgin queen and DPC originated from the colonies in the performance testing. Due to the 35 % of matings assigned to the foreign drones, the estimated inbreeding over 10 generations the genetic variance would be minimal, and the genetic variation would be similar to initial one. The relative genetic gain is expected to be about 1.5 units (Figure 4).



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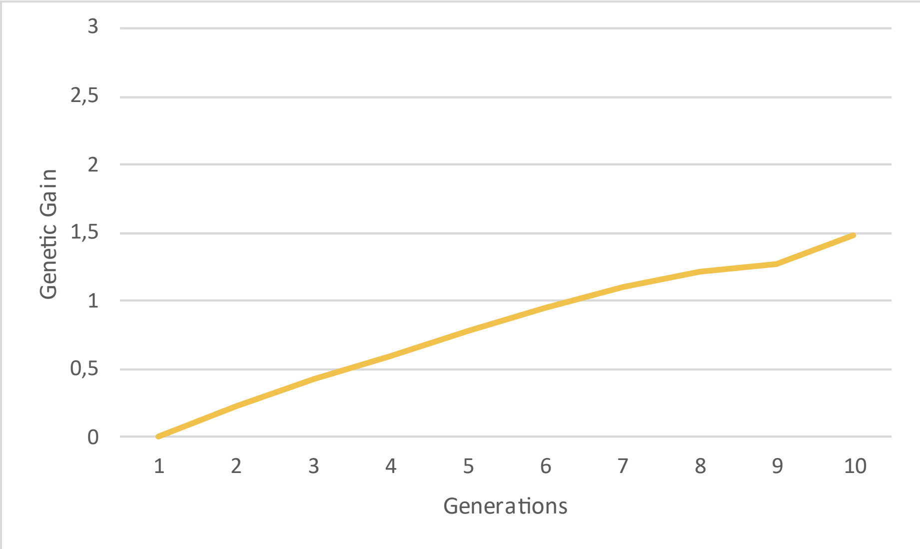


Figure 4. Genetic gain for 10 generations on mainland mating station

The instrumental insemination was considered in the breeding program aiming for fast genetic progress. It was also observed in modelling where the genetic gain was 2.6 units (Figure 5). However, the cost of the genetic gain could be seen in reduction of the initial genetic variance up to 30 % (though the average reduction of 20 iterations was 25 %), and in inbreeding rise to 12 %.

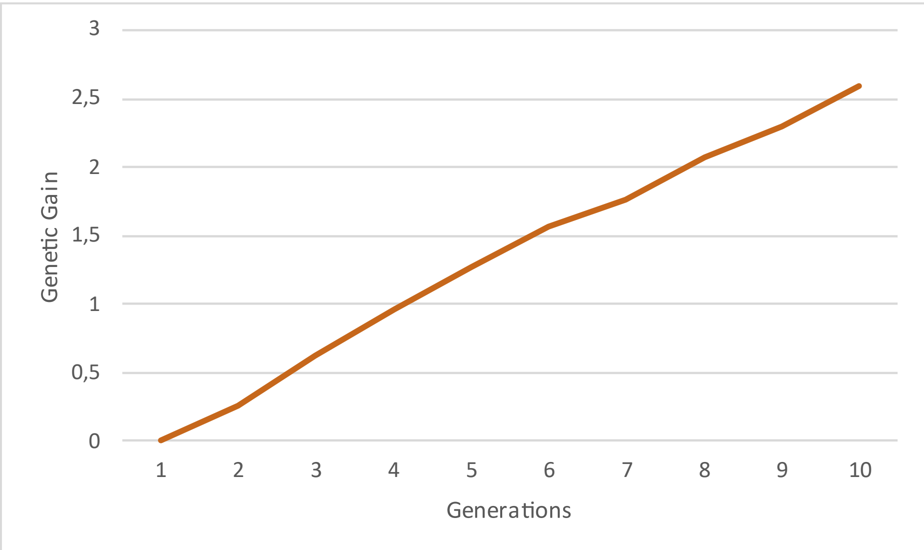


Figure 5. Genetic gain for 10 generations for instrumental insemination



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## Genetically balanced output

Balanced genetic gain can be obtained by engaging both mating control methods in parallel with adjustments in selected queens. The outcome in ten generations showed inbreeding kept under 3 %, genetic variation maintained, and relative genetic gain of 1.9 units. Reducing genetic gain for 0.7 units in comparison with pure instrumental insemination was high, but one should also consider the capacity constraints of the instrumental insemination and the price of mated queen, which was set to be eight times higher than the queen produced on an isolated mating station. Also, when balanced approach would be implemented, the average price of the mated queen would be 45 % higher.

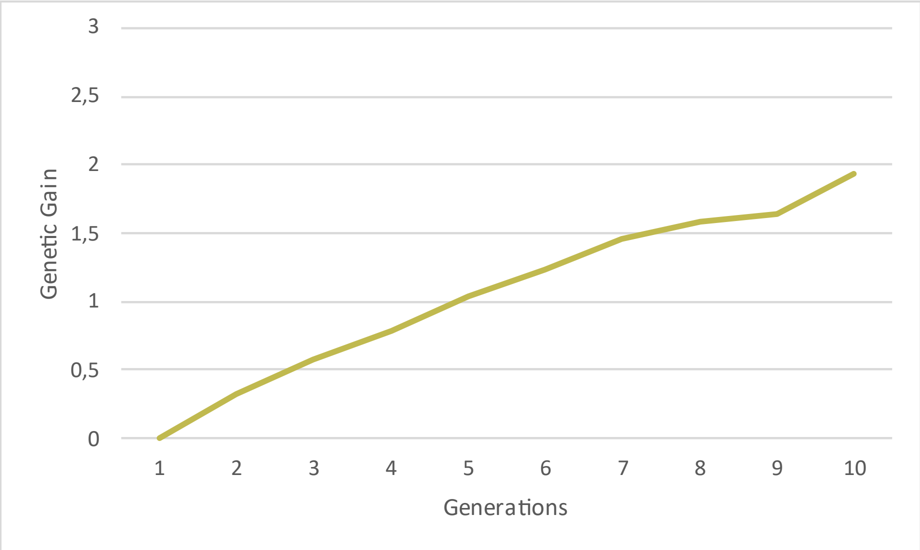


Figure 6. Genetic gain for 10 generations balanced for the inbreeding and genetic variation


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# Slovenia

## Inputs in modelling process

The total population size of honey bees in Slovenia is about 213000 kept by about 10000 beekeepers. Slovenian beekeeping is characterized by very high density of colonies, which makes running a breeding program with mating control difficult. The process also aimed to have a single breeding program, where 1/3 of the queens would be produced. The modelling was prepared for the proposed two methods, alpine high-altitude valley and instrumental insemination. The two concepts differed in reliability and survival rate, but on the other hand the cost rate was equal. Hence, the cost wasn't considered as a contributing parameter. The program is based on the annual use of 100 colonies for queen production and 10 colonies as DPC. The colonies performance testing was set at 600 (direct) and 700 (progeny), which can be a good base for monitoring the population status. The ancestor's information for the dam side would be available for 40000 colonies, while about 400 queens would have full pedigree - e.g. both parental sides are known. Mating control will be used by 10 beekeepers. For the initial phases of the breeding program, there was no plan for genotyping. More details regarding the background of the breeding program are given in Table 3. Input data for the breeding program modelling.

Table 3. Input data for the breeding program modelling

| Parameter | Number |
|---|---|
| Number of honey bee colonies: | 8200 |
| Number of beekeepers | 33 |
| Number of colonies under performance testing | 600+700 |
| Number of beekeepers using performance testing | 30 |
| Number of annually genotyped queens | 0 |
| Annual queen production | 40000 |
| Colonies used for queen production | 100 |
| Number of DPC | 10 |
| **Known ancestors of queens for queen and drone production** | |
| Only dam | 40000 |
| Only sire | |
| Both | 400 |
| Beekeepers in use of mating control | 10 |


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| Mating control in use | |
|---|---|
| **Alpine high-altitude valleys** | |
| Cycles | 4 |
| Virgin queens per cycle | 80 |
| DPC | 10 |
| Patriline success | 70% |
| Losses | 20% |
| **Instrumental insemination** | |
| Cycles | 1 |
| Virgin queens | 100 |
| DPC | 2 |
| Patriline success | 100% |
| Losses | 10% |
| **Estimated cost rate** | |
| Alpine high-altitude valleys | 8 |
| Instrumental insemination | 8 |

## Mating control implications

Alpine high-altitude valley was shown to have very high reliability as reported in D6. However, for the modelling process the reliability used was set to a lower value of 70 %. Parameters specific for the mating station were 4 cycles, where 80 nucs would be used per cycle and 10 DPCs. The losses during the matings were set to 20 % of the virgin queens. In situations, where only this mating control method was used, a genetic gain of 2.1 units could be expected in 10 generations, as seen in Figure 7. The estimated increase of inbreeding in 10 generations would be 3 %, while the genetic variation will be partially maintained at the same level.

The instrumental insemination would be used also in the breeding program, where 100 queens will be inseminated with drones that originated from 2 DPCs. Such an approach would reduce the genetic variation by 15 %, the genetic gain would be 2.3 units in the 10$^{th}$ generation, as shown in Figure 8. The inbreeding in the generation 10 would be 12.5 % higher than in the first generation.



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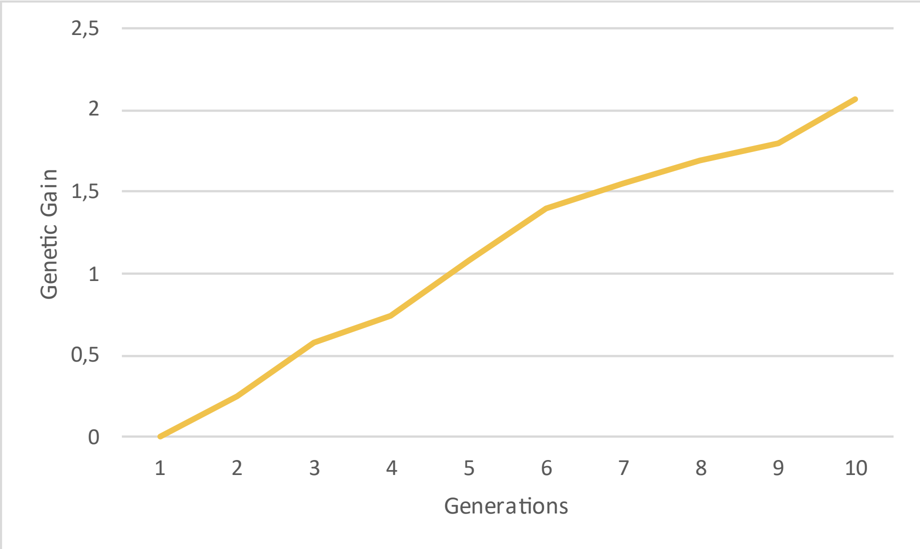


Figure 7. Genetic gain for 10 generations on mating station in Alpine high-altitude valley

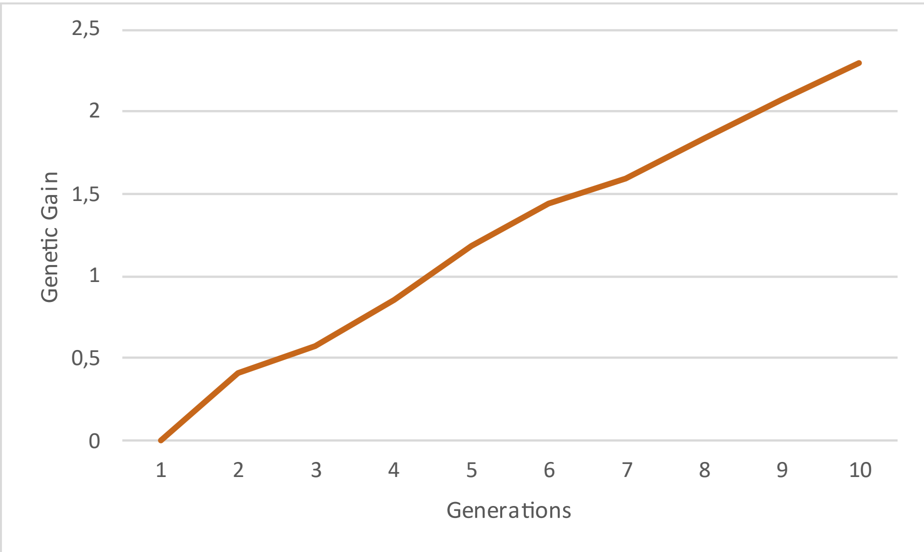


Figure 8. Genetic gain for 10 generations for instrumental insemination



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## Genetically balanced output

Balanced genetic gain could be obtained if both mating control methods would be used in parallel with adjustments in selected queens. The outcome in 10 generations will be an inbreeding under 4 %, genetic variation maintained, and relative genetic gain of 2.1 units (Figure 9). However, the price of the genetically balanced approach comes with a lower genetic gain at around 10 % of that expected in the other two approaches. Nonetheless, it can ensure a long-lasting breeding program and sustainable genetic gain for a long period.

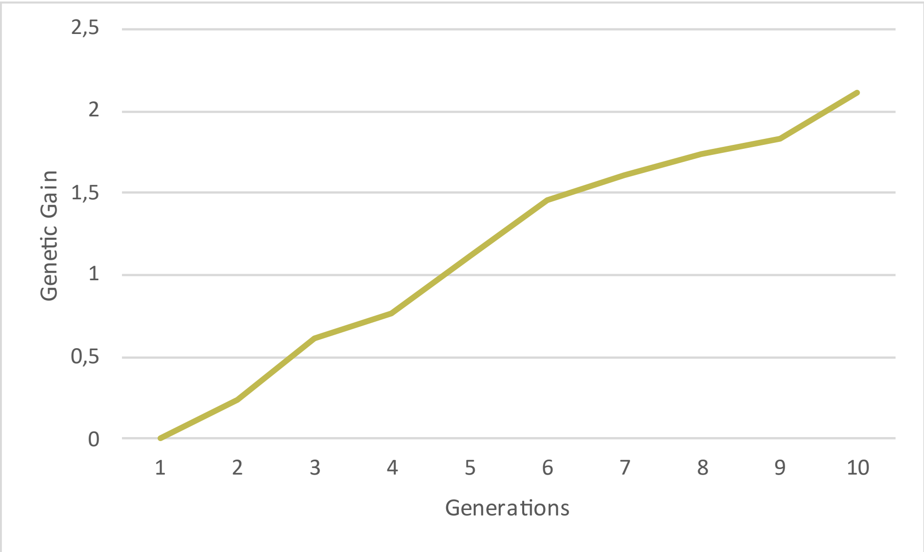


Figure 9. Genetic gain for 10 generations balanced for the inbreeding and genetic variation


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# CONCLUSION

Breeding programs in honey bees can utilize different modalities of mating control, suitable for the specific conditions in the beneficiary countries. Moreover, the different breeding purposes and population size and structure require specific adaptations. Within this deliverable, we project the likely outcomes of different mating control schemes. It is obvious that breeding programs are feasible, and can lead to genetic gain, but the steps should be taken carefully. Genetic improvement is slow but powerful, and the achievements are cumulative. One should note that the genetic gain here is under selection, neglecting the environmental and management conditions, which are hardly predictable for the next 20 years. Also, for the simplicity the modelling included only one moderately inheritable trait, which *per se* is not a completely realistic approach in the breeding program.

In all constellations, the expected genetic gain for 10 generations under balanced model is promising. The inbreeding is an issue particularly when offspring originating from a relatively small number of queens, is used for propagating a large number of virgin queens or is used for DPCs. Before the final decision is taken, a much deeper analysis of the capacities, structure, costs, and approaches is compulsory.



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