Contributed paper

HOW ARE YOU FEELING, GIRLS? – BEHAVIOURAL TRAITS AS EMERGENT PROPERTIES OF THE COMMUNITY

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SUMMARY

While individual properties arising from interactions between individuals can have a significant impact on trait expression across a vast range of species in both animals and plants, nowhere does this become more immediately apparent than in trait definition and assessment of honeybees.

Due to the eusocial nature of these insects, phenotypic observations are, for the most part, limited to the superorganism, a complex community of up to 80,000 honeybees. Individual assessment of the behaviour and physiology of both worker and queen bees is possible (e.g. aggression in workers, egg-laying in queens) but of limited usefulness since the opportunity for their expression in the context of the honeybee colony might be limited or favoured via their interaction. As a result, a different overall phenotype can be expressed by the colony than would be expected based on the individual assessment.

By including a “community” component in our understanding of trait expression, we can explore the many layers of individual trait expression which contribute to worker brood viability in honeybee colonies and their intersection with both genetic and environmental factors. The inclusion of community aspects allows us to include fundamentally separate aspects that cannot be explained or captured by traditional models defining phenotypes as a result of G, E and GxE.

INTRODUCTION

Great strides have been made in genetic improvement of plants and animals over the past decades, but some challenges in genetic evaluations remain, especially around behavioural traits (Chang *et al.* 2020). Complex phenotypes and a limited understanding of all the factors influencing the expression of traits hinder the adequate parcelling and attribution of variation, both genetic (G) and non-genetic or environmental (E). In the classic partitioning of variation into components of genetic and environmental variation, variation due to interactions of the individuals with the external world is often lost in the environmental component or masked (Foris *et al.* 2018). This was partially overcome by the introduction of an interaction component between the genetics of an individual and the environment, thereafter referred to as GxE (Falconer 1952). However, the G+E+GxE framework neglects the role that social interactions as well as underlying factors such as population density and population structure play for the realisation of genetic potential.

Phenotypic variation is not fully explained by current methods, although additional aspects like epigenetics can contribute to our understanding of the occurrence of variation (Triantaphyllopoulos *et al.* 2016). Contributors to phenotypic variation that can be considered both “environmental” and “social” have been recognised in the field of animal breeding since the 1970s (Willham 1972) and have found entry into genetic evaluations in some species in the form of maternal effects (Solé *et al.* 2021). However, a large proportion of community-driven factors that contribute to variation (e.g. competition, genetic makeup of the population) are still often either considered completely environmental or entirely due to individual genetics.

METHODS AND DISCUSSION

Understanding individual contributions to the superorganism. As eusocial insects, honeybees are limited to a small number of reproductively active females, the queens, who are supported by functionally sterile female worker bees at a ratio from 1:5,000 to 1:80,000. This puts them in a unique situation where phenotypic observations are, for the most part, limited to the superorganism, a complex community of tens of thousands of honeybees, while selection can only act on the core individual, the queen.

While some traits, such as the reaction to *Varroa* mite infestations, can be assessed in individual workers (e.g. Currie and Tahmasbi 2008), these observations are of limited usefulness since they might not find an opportunity to be expressed by the individual in the context of the entire colony.

Honeybee traits are often the result of multiple populations within a colony working together in cohorts of sisters of a similar age, which means that the exact expression of any trait relies on the performance of hundreds of individuals, each of them with an individual response threshold that triggers behaviours which contribute to the observed trait (Beshers and Fewell 2001). An approach trying to integrate studies of the behavioural, physiological and neurobiological aspects of division of labour developed a push-pull model explaining the relationship between different castes and their respective ages under natural conditions (Johnson 2010).

The GCE model in theory. Assuming that division of labour impacts on trait expression, a new approach is needed to interpret observations of honeybee performance before these can be used as the basis of genetic evaluations. The required model must allow for the consideration of modifiers that contribute to the outcomes for an individual worker and her life history. These modifying factors can be split into two classes: *effectors* and *responses*.

To fully explain the expression of the genetics of an individual bee, *effectors* are both the community that the individual partakes in and the environment they live in. These two effectors are necessary for the expression of an individual's genetics but exist largely independently from an individual bee's life or are only slightly influenced by her individual contribution (see circles in Figure 1).

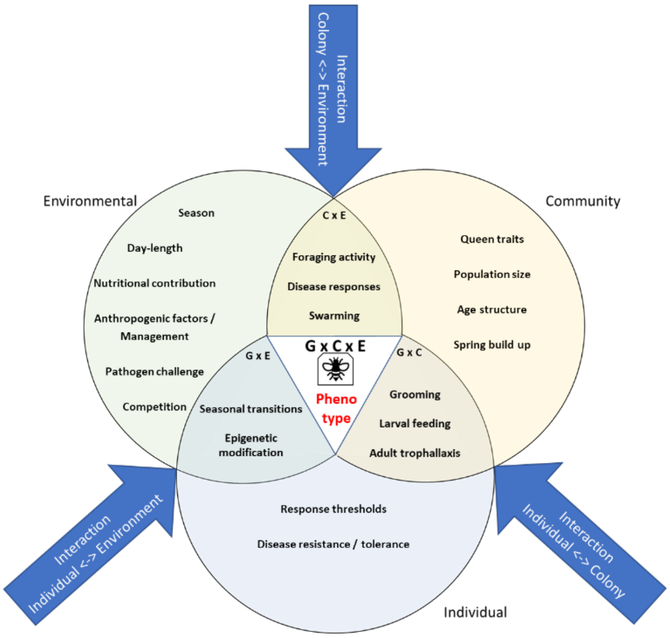

Figure 1. Intersections between individual genetic basis, environmental aspects and community in honeybee trait expression

The *responses* that an individual show as a result of their interaction with the effectors are adaptable outcomes of said interactions which are specific to the individual, since they are based on their individual response thresholds. These responses serve as measurable phenotypic outcomes that add to the individual's life history and in turn contribute to the phenotype of the superorganism. Responses arise from the interaction of individual genotypes with the environment (GxE), and the other members of the community (GxC) which is influenced by environmental effects on the community through individual interactions with the environment (CxE).

Applying the GCE model to worker brood viability. In applying the GCE framework to a particular trait, contributors to trait expression can be broken down and attributed directly to their source, rather than having to rely on a theoretical ability to control for these contributors.

Worker brood viability, the percentage of brood cells that are capped over in a patch of brood of similar age, is a complex phenotype driven by numerous factors.

Queen egg laying rate (see Figure 2, "Community") establishes an upper limit for the number of worker brood cells that can be capped at any time, since it determines how many eggs are developing within the roughly 10-day time frame that covers larval and pupal development under wax caps, the only time when brood viability can be readily observed. While queen laying traits are hard to observe without somehow limiting their expression (e.g. by supplying a limited number of cells in a confined area of the hive for a set number of hours), they can be measured. However, they do not present an adequate representation of the colony's ability to live up to the upper limit they present.

Brood care is a core part of the inner workings of a honeybee hive, and its success depends on the availability of capable nurse bees (who need to be of the correct age to be able to produce larval food) as well as the availability of food. Food can be stored as nectar, honey and bee bread, which can be assessed by the beekeeper and would reduce its function as an effector to an environmental effect. However, at times of high food availability outside the hive and depleted stores, the standard situation in spring, food resources can be directly distributed by worker bees foraging outside of the colony. This both brings in an aspect of a community dependency as well as a complication for the observation and quantification of the environmental effectors by the beekeeper since these transitional food sources are almost impossible to assess.

The individual genetic factors which contribute to observed brood viability apply to the larvae in development at the time of observation of brood viability, and include genetic disease resistance, response thresholds for nutritional and environmental factors like the ability to tolerate variation in brood comb temperature, and the allele status at the honeybee sex determination locus *csd*, which homozygosity at which can result in non-viable diploid male larvae see Figure 2, "Individual").

Limitations of the model. While the GCE model presented here can serve to identify contributors to honeybee trait expression that were previously impossible to determine in the context of the superorganism, it cannot be readily applied in the interpretation of honeybee performance to improve on genetic evaluations with the use of traditional observational data collected by beekeepers, as this information is not of sufficient granularity to generate insights into the community effector. However, with the increased use of beehive telemetry in routine beekeeping, datasets are becoming available which will allow the application of the GCE model to hive performance data in order to tease apart environmental and community contributions to superorganism phenotypes and thus fully define the genetic contribution and increase accuracy in honeybee genetic evaluations.

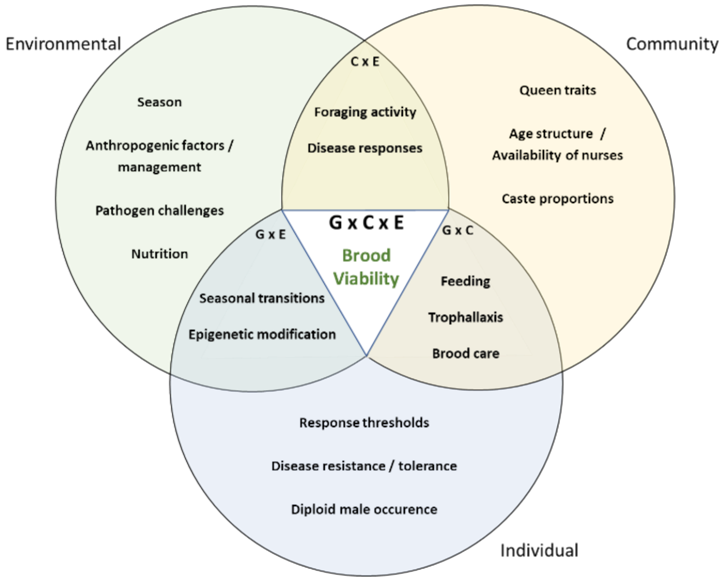

Figure 2. Worker brood viability as an emergent property of the interaction of Genotype, Community and Environment

CONCLUSIONS

Treating phenotypes of the honeybee superorganism as emergent properties of the interaction between genetics, environment, and the community within the colony can help define contributors to observed variation and strip away variation which has previously clouded our understanding of the genetic effect on honeybee performance.

While the framework can be most readily applied in eusocial insects, it is likely to have applications in other livestock species, e.g. in defining the effects of competition on performance and survival, as well as in plant production systems where competition for natural resources and space cannot be avoided via translocation.

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REFERENCES

Beshers S. N. and Fewell J. H. (2001) *Annu. Rev. Entomol.* **46**: 413.
Chang Y., Brito L.F., Alvarenga A.B., Wang Y. (2020) *Anim. Front.* **10**: 29.
Currie R.W., Tahmasbi G.H. (2008) *Canadian J. Zool.* **86**: 1059.
Falconer D.S. (1952) *The American Naturalist* **86**: 293
Foris B., Zebunke M., Langbein J., Melzer N. (2018) *PLoS ONE* **13**: 1.
Solé E., Ros-Freixedes R., Tor M., Reixach J., Pena R.N., Estany J. (2021) *PLoS ONE* **16**: 1.
Triantaphyllopoulos K.A., Ikonomopoulos I., Bannister A.J. (2016) *Epigenetics Chromatin* **9**: 1.
Willham R.L. (1972) *J. Anim. Sci.* **35**: 1288.