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ORIGINAL RESEARCH ARTICLE

Association of single nucleotide polymorphisms to resistance to chalkbrood in *Apis mellifera*

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Summary

Chalkbrood is a honey bee brood disease that often affects colonies that are already under stress. Control of the disease can be as simple as ensuring adequate ventilation and food sources or using clean beekeeping equipment. When the infection goes unchecked, however, the overall health and productivity of the colony is greatly decreased. Some strains of honey bees seem to be more troubled by the disease than others. Efforts to control rampant infections have not been widely accepted or successful. Identifying a genetic basis for resistance in the affected larvae would be useful for breeding for improved resistance in bee populations. We here show a statistically significant association between larval chalkbrood resistance and a genomic locus. Selective breeding for larval resistance can probably work in concert with breeding for desirable characteristics such that chalkbrood can become a negligible disease among managed colonies.

Asociación de polimorfismos de nucleótido único con la resistencia a Ascosferosis en *Apis mellifera*

Resumen

La Ascosferosis es una enfermedad de la cría de la abeja de la miel, que a menudo afecta a colonias que ya están bajo estrés. El control de la enfermedad puede ser tan simple como asegurar una ventilación adecuada y fuentes de alimentos o el uso de un equipo apícola limpio. Sin embargo, cuando no se toman medidas contra la infección, el estado general de salud y la productividad de la colonia se ve considerablemente mermado. Algunas poblaciones de abejas parecen presentar mayores problemas por la enfermedad. Los esfuerzos para el control de la proliferación de infecciones no han sido ampliamente aceptados o exitosos. La identificación de una base genética de resistencia en las larvas afectadas podría ser útil en la reproducción para la mejora de la resistencia en las poblaciones de abejas. Aquí se muestra una asociación estadísticamente significativa entre resistencia a la Ascosferosis y un locus genómico. La reproducción selectiva para resistencia de las larvas puede realizarse en conjunto con la reproducción para características deseables, de manera que la Ascosferosis pueda llegar a convertirse en una enfermedad insignificante entre las colonias manejadas.

Keywords: chalkbrood disease, honey bee, *Apis mellifera*, *Ascosphaera apis*, SNP association, marker assisted breeding

Introduction

The honey bee fungus *Ascosphaera apis* causes the development of chalkbrood, an infection that attacks larval honey bees between three and four days old(Flores *et al.*, 1996). The infection process, initiated by the ingestion of fungal spores through contact with contaminated nurse bees, occurs by germination of spores in the gut which eventually populate the lower abdomen and break through to the outer surface of the larva (Heath, 1982b).The infection process kills through physical and physiological damage to the larva (Glinski and Buczek, 2003). The dead larvae appear "mummified" due to the eventual encapsulation of the corpse with a thick layer of white mycelium which desiccates over time and takes on a chalky appearance, hence "chalkbrood".

A wide range of treatments have been used to attempt to minimize or prevent the development of the disease, such as chemical pesticides, clean beekeeping practices, and natural inhibitory products, but little progress has been made in chalkbrood control.

Chemical agents pose risks to the general health of colonies and may contaminate the honey crop with residual compounds (Hornitzky, 2001). Sanitary beekeeping practices have proved ineffective, as spores can remain viable for 15 years and can be found in honey, comb wax, and pollen (Flores *et al.*, 2005; Heath, 1982a). Natural controls such as antifungal compounds in essential oils (Davis and Ward, 2003) and fungal-inhibitory mechanisms in bacteria (Gilliam *et al.*, 1997, Gilliam *et al.*, 1988) are showing promise in laboratory settings but have not been adopted by the industry.

Altering the genetic stock through selective breeding may decrease the prevalence or severity of infections. The normal colony response to chalkbrood is the removal of larval corpses through general hygienic behaviour (Invernizzi *et al.*, 2011). Hygienic behaviour is highly valued and subject to selection by beekeepers for responses to *Varroa destructor* mites and other brood-specific insults. In the case of fungal diseases, however, the removal of infected "mummies" may, in fact, lead to the widespread distribution of spores throughout the colony. Alternatively, identification and selective breeding for genes responsible for resistance to chalkbrood in the larvae themselves (Invernizzi *et al.*, 2009) may be the most effective means of control. To address a genetic basis of larval resistance, larvae were produced for a backcrossed resistant/susceptible population, brood comb was inoculated with spores, and infection was allowed to initiate. Larvae that exhibited fungal mycelia on the lower abdomen were classified as susceptible as opposed to healthy resistant brood. DNA was extracted from susceptible and resistant samples and subjected to SNP (single nucleotide polymorphism) analysis. We demonstrate through statistical associations between the resistant and susceptible phenotypes with marker genotypes that there is a genetic basis for chalkbrood resistance, and a potential to selectively breed for resistance in managed colonies.

Materials and methods

Population development

During the summer of 2004 in Baton Rouge, LA, USA, 33 colonies of a variety of commercial stocks of honey bees and 38 colonies of Russian honey bees were inoculated with chalkbrood spores generated from 1 g of pulverized mummified larval corpses mixed with 150 ml of a 1:2 (wt:v) sugar water solution. The suspension was allowed to reconstitute for 30 min then sprayed onto brood comb and returned to the colony. Larvae were inoculated and evaluated for the initiation of chalkbrood infection 6, 13 and 20 days after baseline determinations. Drones reared from resistant commercial colonies were used for single-drone-insemination of queens reared from susceptible Russian colonies. F1 queens were reared and back-crossed by single-drone-insemination to drones from susceptible Russian colonies. A single fecund queen was chosen as the larval source for subsequent laboratory-based evaluation of resistance.

Evaluation of resistance

A single-drone-inseminated queen was caged on brood comb which had been previously conditioned by worker bees. The queen was then transferred to a new area of conditioned comb and the aged cohort worker eggs were isolated to prevent further egg deposition on the experimental brood frame. A suspension of spores was generated by mixing 1 g of pulverized mummified larval corpses with 150 ml of a 1:2 (wt:v) sugar water solution and allowed to reconstitute for 30 min. The spore suspension was sprayed onto the experimental brood frame containing <4 d old larvae, incubated at 35°C, 70% RH (RH) for 1 h and returned to the colony for 48 h to allow cell capping. The brood frame was then incubated at 18°C for 2 h to promote chalkbrood development, then 35°C, 70% RH for a minimum of 48 h in the absence of worker bees to prevent hygienic removal. Brood cells were uncapped and examined three times daily for the presence of chalkbrood mycelia on the lower abdomen. Susceptible larvae/pupae (those with mycelial development) were removed and individually frozen for subsequent DNA extraction. Resistant individuals were all those remaining that failed to exhibit mycelial development on three consecutive examinations after the last susceptible larva was observed. Resistant larvae were individually collected approximately 10 days after inoculation (24 h after the last susceptible individual was identified) for subsequent DNA extraction.

SNP analysis

Statistical associations between SNPs and the bimodal resistance trait were determined by several methods using mapping software as well as manual statistical analyses. LOD scores were determined by both the Haldane and Kosambi mapping functions in WinQTL Cartographer v2.5 (Wang *et al.*, 2011) by Single Marker Analysis (SMA) and Interval Mapping (IM) set up for a backcrossed population. Data input for the software accounted for the bimodal trait by assigning a binary phenotype score and assigned an artificial map position based on marker order per chromosome rather than true genetic position. Additionally, a manual determination of association *p*-values was performed for each marker and binary phenotype score by Student's *t*-test.

Results

Phenotyping for chalkbrood resistance at colony level and in the mapping population

Following a baseline assessment of pre-inoculated colonies, resistant colonies failed to develop chalkbrood over the 20 day evaluation, whereas susceptible colonies developed a range of infection in larvae. While both Russian and commercial colonies exhibited resistant and susceptible phenotypes, Russian susceptible colonies developed much more severe infections with up to 6% of larvae infected whereas the most infected commercial colony exhibited less than half that (Table 1).

Table 1. Evaluation of chalkbrood resistance at colony level in two stocks of honey bees.

	Russian	**Commercial**
Number of colonies evaluated	38	32
Number (per cent) resistant colonies	16 (42)	13 (41)
Maximum % infected larvae in a susceptible colony	6.09	2.4
Minimum % infected larvae in a susceptible colony	.001	.01

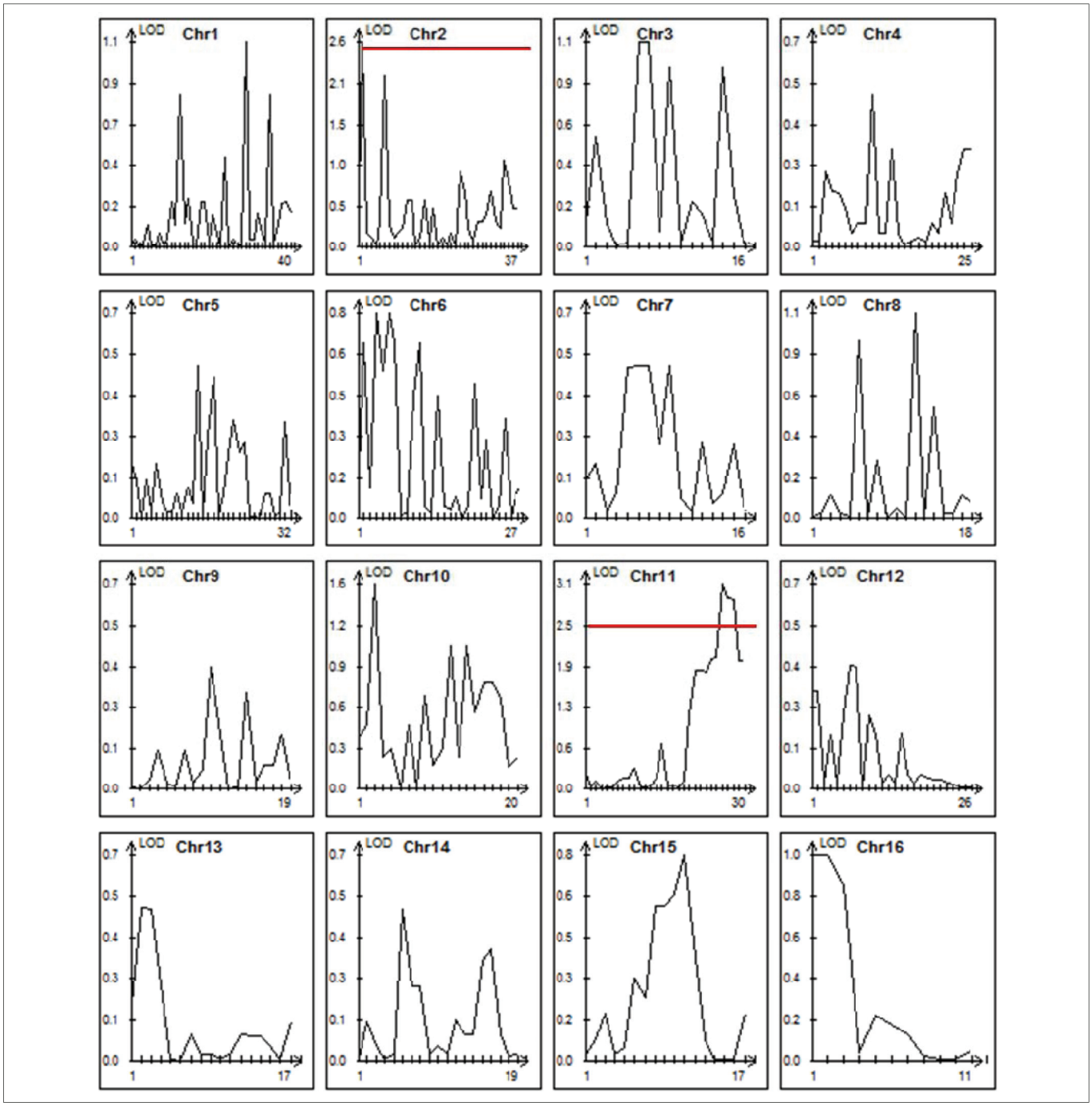

Fig. 1. SNP association peaks for chalkbrood resistance in honey bees. QTL Cartographer single marker analysis identifies associations between SNP and the chalkbrood resistance trait per chromosome. A LOD score threshold value of >2.5 (red line) shows that only associations on chromosomes 2 and 11 suggest a genetic basis for resistance. The x-axis for each graph shows the number of markers mapped to each chromosome.

Table 2. Significance of associating peaks from Single Marker Analysis of SNP markers and chalkbrood resistance in honey bees.

SNP ID	**AMB-000469637**	**est8741**	**est8750**	**est8764**
Chromosome	2	11	11	11
Genomic position (Kb)	9.7	13636.1	13691.7	13698.3
Win QTL Cartographer LOD score	2.58	3.14	2.91	2.87
Student's t-test p-value	.00062	.000154	.000279	.00022
Significant following Bonferroni correction	No	Yes	No	Yes

Methods for inoculating the mapping population achieved appropriate ratios of susceptible versus resistant larvae. A total of 582 larvae were inoculated and scored for development of infection of which 380 (65%) were scored as resistant, and 202 (35%) were susceptible. The ratio suggests that the experimental design and choice of queen/ drone sources were sufficient for generating a backcross mapping population. The last susceptible larva was identified approximately nine days after inoculation, and no more mycelial development was observed for the subsequent 24 hours suggesting that the remaining larvae were resistant.

SNP associations

Ninety-two individuals from each of the resistant and susceptible groups were genotyped. Filtering non-polymorphic SNPs from the 1,536 SNPs analysed resulted in 469 polymorphic markers from the backcrossed population useful for mapping. Of those, 370 were well mapped to chromosomes 1 through 16 and used for the association mapping. Single marker analysis (Fig. 1) and interval mapping (data not shown) both identified the same two genomic loci (chromosomes 2 and 11) with suggestive associations (LOD >2.5) to the resistance trait. Using a Bonferroni correction (α/n where $\alpha \leq 0.1$, $n = 370$ markers), only the associations of two SNPs (est8741 and est8764) on chromosome 11 persisted (Table 2). An additional SNP adjacent to the significant SNPs suggests that a QTL exists in the genomic region, however that SNP failed to pass the significance threshold determined by the Bonferroni correction. Rough false discovery rate [$\alpha(m+1)/2m$, where $\alpha \leq 0.1$, $m = 370$ independent markers] was estimated at 0.05. The approximate 1.5 Mb interval surrounding SNPs est8741 and est8764 (defined by the >2.5 LOD score output from QTL Cartographer) contains over 200 known or hypothetical genes (Table 3). Evaluation of those genes did reveal potential candidate genes involved in chalkbrood resistance including a potential member of the NF-kappa-B immune response pathway ("NF-kappa-B repressing factor-like"). Several biochemical pathways are represented that have previously been shown to be transcriptionally regulated in response to chalkbrood infection (Aronstein *et al.*, 2010). In particular, members of the chitin biosynthesis pathway, ubiquitin pathways, second messenger pathways, and oxidative stress response pathways lie in close proximity to the significant SNP, suggesting a functional role for one (or more) of the proteins expressed from the genomic region in regards to chalkbrood infection.

Table 3. Annotated honey bee genes (excluding hypothetical genes) within an approximate 1.5Mb interval surrounding the suggestive SNPs est8741 or est8764. Genes and positions are listed according to Amel 4.5 (honey bee genome sequencing project) via NCBI map viewer.

Start Position	**Stop Position**	**Symbol**	**Description**
12987209	13054424	fng	fringe glycosyltransferase
13055026	13061163	LOC408319	lethal (3) malignant brain tumour ortholog
13069400	13082096	CTL2	C-type lectin 2
13082815	13091479	Madm	MLF1-adaptor molecule
13092212	13096786	LOC724877	integral membrane protein GPR155-like
13103200	13108226	Mlf	myelodysplasia/myeloid leukemia factor
13109867	13112197	LOC552769	ubiA prenyltransferase domain-containing protein 1 homolog
13112935	13114315	LOC100577344	gametocyte-specific factor 1-like
13114975	13120524	UBE4B	ubiquitination factor E4B (UFD2 homolog, yeast)
13121556	13122446	LOC727234	cytidine deaminase-like

Table 3 Cont'd. Annotated honey bee genes (excluding hypothetical genes) within an approximate 1.5Mb interval surrounding the suggestive SNPs est8741 or est8764. Genes and positions are listed according to Amel 4.5 (honey bee genome sequencing project) via NCBI map viewer.

Start Position	Stop Position	Symbol	Description
13122409	13130770	Tor	target of rapamycin
13132388	13134768	ABC10	ATP-binding cassette, sub-family B, member 10
13135465	13138279	PPP4R2r	protein phosphatase 4 regulatory subunit 2-related protein
13140307	13141793	LOC551313	calcineurin subunit B type 2-like
13142789	13144233	Ial	serine/threonine-protein kinase Ial
13144052	13147027	LOC411505	putative tRNA pseudouridine synthase Pus10-like
13149379	13152575	LOC552714	conserved oligomeric Golgi complex subunit 2-like
13153398	13155683	LOC413822	ubiquitin-conjugating enzyme E2 L3-like
13156098	13163058	cul-3	cullin 3
13164280	13166552	Idh	isocitrate dehydrogenase
13171644	13178766	LOC410108	zinc finger protein 729-like
13179157	13194087	LOC727221	vacuolar protein sorting-associated protein 13C-like
13195673	13210736	osa	trithorax group protein osa
13211443	13214371	LOC409329	mannose-1-phosphate guanyltransferase beta
13213926	13217693	LOC100576647	separin-like
13217754	13220370	mmy	mummy
13226112	13230379	LOC725781	coiled-coil domain-containing protein 132-like
13231804	13235265	LOC409241	prohormone-4
13239105	13241368	LOC411861	NF-kappa-B-repressing factor-like
13242247	13243537	mRpS2	mitochondrial ribosomal protein S2
13243541	13245120	LOC411863	dehydrogenase/reductase SDR family member 7-like
13252560	13256690	LOC409634	MVPVPVHHMADELLRNGPDTVI-containing neuropeptide
13264266	13266384	Syx18	syntaxin 18
13266425	13268586	cope	epsilon subunit of coatomer protein complex
13271078	13274350	Gld2	poly(A) RNA polymerase gld-2 homolog A
13274443	13281189	Upf1	regulator of nonsense transcripts 1
13282498	13294025	bib	big brain
13296482	13300600	Gli	gliotactin
13322972	13326813	polo	serine/threonine-protein kinase polo
13331304	13339074	LOC551061	multidrug resistance-associated protein 4-like
13339762	13346625	LOC725051	probable multidrug resistance-associated protein lethal(2)03659-like
13355947	13363393	SF3B3	splicing factor 3b, subunit 3, 130kDa
13366780	13369518	LOC412409	mitochondrial import inner membrane translocase subunit TIM44-like
13370768	13384788	hyd	E3 ubiquitin-protein ligase hyd
13384661	13388688	bun	bunched
13389250	13391932	LOC411554	Williams-Beuren syndrome chromosomal region 16 protein homolog
13400004	13405032	Nup133	nucleoporin 133
13405650	13412271	Aats-val	valyl-tRNA synthetase
13411354	13417034	LOC409184	DENN domain-containing protein 1A-like

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Start Position	Stop Position	Symbol	Description
13417405	13418889	LOC413145	eukaryotic translation initiation factor 3 subunit F-1
13418816	13420652	Arp11	actin related protein 11
13421027	13422119	Rpn12	Rpn12 protein
13425656	13427756	LOC726151	probable 39S ribosomal protein L49, mitochondrial-like
13505626	13506905	Grx2	glutaredoxin 2
13568496	13568595	Mir928	microRNA mir-928
13570281	13576793	LOC100576519	TLD domain-containing protein KIAA1609 homolog
13587039	13589963	LOC552832	glycine N-methyltransferase-like
13591610	13606887	LOC726408	zinc finger protein 706-like
13595522	13598517	Snap	soluble NSF attachment protein
13598935	13601345	LOC100576947	uncharacterized protein C16orf73 homolog
13603141	13605972	PLA2-2.4	phospholipase A2-2.4
13609974	13613955	Pat1	protein interacting with APP tail-1
13614003	13617612	LOC726432	rab GTPase-binding effector protein 1-like
13618618	13622205	LOC409941	zinc finger FYVE domain-containing protein 1-like
13630580	13637735	LOC409942	interference hedgehog-like
13751291	13760579	LOC551454	voltage-dependent calcium channel subunit alpha-2/delta-3-like
13762211	13773135	Nup98	nucleoporin 98kDa
13773976	13781776	LOC551500	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta isoform-like
13782653	13787595	LOC726523	protein white-like
13788545	13792034	st	scarlet
13809832	13815577	LOC726455	mutS protein homolog 4-like
13816051	13824328	LOC408332	zinc finger protein 236-like
13823606	13826196	LOC410286	probable tRNA (uracil-O(2)-)-methyltransferase-like
13826330	13827321	LOC726576	UPF0631 protein-like
13827254	13830352	LOC100576660	FAD synthase-like
13857219	13880863	HDAC4	histone deacetylase 4
13881421	13883481	LOC410283	transmembrane protein 222-like
13890810	13928978	Syx1A	syntaxin 1A
13893827	13897081	LOC100576279	prolow-density lipoprotein receptor-related protein 1-like
13897872	13901192	LOC408328	elongation factor Tu, mitochondrial-like
13901091	13903992	ran	GTP-binding nuclear protein Ran
13928286	13939436	LOC408324	t-cell immunomodulatory protein-like
13930707	13932827	Cdk5	cyclin-dependent kinase 5
13939939	13949575	Picot	inorganic phosphate cotransporter-like protein
13954387	13954997	Dctn5	dynactin subunit 5
13961828	13968946	cv-2	crossveinless 2
13974235	13992933	Pkc	protein kinase C
13980511	13999724	LOC410272	low affinity cationic amino acid transporter 2-like

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13999819	14003421	lds	transcription termination factor 2
14003895	14011143	LOC410274	transcription elongation factor SPT6-like
14023912	14025419	LOC724155	peptide chain release factor 2-like
14024112	14032522	chif	chiffon
14033565	14035331	loj	logjam
14034951	14044223	Exn	ephexin
14046082	14047635	LOC100577449	PMS1 protein homolog 1-like
14049974	14056575	CkIIalpha	casein kinase II alpha subunit
14058373	14060753	LOC409766	ubiquitin-like modifier-activating enzyme 5-like
14060092	14062181	LOC409765	actin-related protein 13E ortholog
14062869	14077468	LOC413097	dynein heavy chain at 62B ortholog
14079309	14083874	muskelin	muskelin protein
14083269	14088674	LOC413096	ubiquitin-protein ligase E3C-like
14089929	14092340	LOC724878	GalNAc-4-sulfotransferase
14096776	14099264	LOC724998	solute carrier family 2, facilitated glucose transporter member 10-like
14121427	14127718	LOC413092	FERM, RhoGEF and pleckstrin domain-containing protein 1-like
14137965	14144599	LOC413091	serrate RNA effector molecule homolog
14154565	14154636	Trnaa-ugc	transfer RNA alanine (anticodon UGC)
14162048	14162967	Lsm7	U6 snRNA-associated Sm-like protein LSm7
14167872	14171077	Scp1	sarcoplasmic calcium-binding protein 1
14172789	14183566	LOC552375	niemann-Pick C1 protein-like
14185271	14187963	LOC725497	peptidyl-prolyl cis-trans isomerase-like 4-like
14190977	14212204	lig3	DNA ligase III
14211870	14219357	LOC725616	prohormone-1
14231840	14234549	LOC413087	tRNA (uracil-5-)-methyltransferase homolog A-like
14234220	14237415	LOC552254	bifunctional polynucleotide phosphatase/kinase-like
14237839	14242821	LOC552231	probable RNA-binding protein 19-like
14264105	14275401	Actn	alpha actinin
14276153	14278141	LOC100577520	uncharacterized protein C19orf47 homolog
14281695	14285331	LOC412154	oligopeptidase A-like
14284937	14286565	Syx8	syntaxin 8
14286376	14290937	LOC409373	transmembrane protein 184B-like
14295897	14298822	Taf6	transcription initiation factor TFIID subunit 6
14299925	14302574	LOC552695	RCC1 and BTB domain-containing protein 1-like
14304255	14306765	LOC408321	RCC1 and BTB domain-containing protein 1-like
14309460	14332477	AChE-1	acetylcholinesterase 1
14344462	14400342	LOC100578939	single Ig IL-1-related receptor-like
14397309	14407677	LOC410269	probable multidrug resistance-associated protein lethal(2)03659-like
14408501	14432061	LOC100578976	biglycan-like

Table 3 Cont'd. Annotated honey bee genes (excluding hypothetical genes) within an approximate 1.5Mb interval surrounding the suggestive SNPs est8741 or est8764. Genes and positions are listed according to Amel 4.5 (honey bee genome sequencing project) via NCBI map viewer.

Start Position	Stop Position	Symbol	Description
14435470	14437735	LOC552746	epidermal retinol dehydrogenase 2-like
14437694	14443166	SYMPK	symplekin
14443625	14445452	LOC726522	ketohexokinase-like
14445647	14445720	Trnai-aau	transfer RNA isoleucine (anticodon AAU)
14448456	14448528	Trnar-acg	transfer RNA arginine (anticodon ACG)
14459385	14462983	LOC726579	cleft lip and palate transmembrane protein 1-like protein-like
14462363	14466690	sec5	exocyst complex component 2
14467588	14471612	Akt1	RAC serine/threonine-protein kinase
14471228	14473678	LOC413429	iron/zinc purple acid phosphatase-like protein-like
14524591	14532824	stnB	stoned B
14533472	14544448	LOC552804	tubulin-specific chaperone cofactor E-like protein-like
14579997	14581511	LOC409978	proteasome subunit beta type-2-like
14581561	14583937	LOC409870	minor histocompatibility antigen H13-like
14588352	14590733	LOC413336	ADP-dependent glucokinase-like
14600520	14604458	mio	WD repeat-containing protein mio
14610389	14616819	Pfrx	6-phosphofructo-2-kinase
14616906	14618447	Gas41	YEATS domain-containing protein 4
14621116	14622306	LOC100578302	solute carrier family 2, facilitated glucose transporter member 3-like
14628429	14642632	Cht3	chitinase 3
14646926	14650819	Pitslre	serine/threonine-protein kinase PITSLRE
14651702	14658625	LOC551449	tyrosine-protein phosphatase non-receptor type 23-like
14659282	14663233	LOC409265	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A-like
14664407	14670402	NUP205	nucleoporin 205kDa
14671432	14675226	LOC726904	NF-X1-type zinc finger protein NFXL1-like
14675714	14679337	LOC551493	pre-mRNA-processing factor 6-like
14681076	14689886	cib	ciboulot
14695104	14701266	NFRKB-like1	nuclear factor related to kappaB binding protein-like 1
14701708	14710365	Ip3k	inositol 1,4,5-triphosphate kinase

Discussion

Chalkbrood infection tends to be seasonally activated by low temperatures or during times of high stress. Colonies that are highly susceptible to chalkbrood are, however, susceptible regardless of the environmental conditions (Heath, 1982b). For this reason, we were able to develop a segregating mapping population for chalkbrood resistance during the summer when chalkbrood is generally rare. By doing so, we ensured that the larval susceptibility was genetically controlled and not a passive environmental insult response.

Our work identified one suggestive genetic association for chalkbrood resistance. Whilst many traits of interest in honey bees are probably multi-genic, including the hygienic removal of chalkbrood corpses, the nature of infection by *A. apis* suggests that few genes would be involved in the larvae themselves. Because the disease is relatively new to honey bees (~100 years) (Heath, 1985) and hygienic behaviour is usually sufficient to combat widespread chalkbrood infection, there is probably little genetic diversity specific for chalkbrood response within bee populations. It could, therefore, be expected that chalkbrood resistance within the larvae is controlled by a single or very few genes. Additionally, beekeeper-mediated selection for chalkbrood resistance through re-queening has been effective as a

control, suggesting a simple system of resistance. If multiple genes were responsible for resistance, more re-queening would be necessary as an increase in variation and genetic combinations that could deem colonies susceptible would be exhibited within populations.

Several genes are in close proximity to the SNP associated with resistance that function in pathways already shown to respond during chalkbrood infection (Aronstein *et al.*, 2010). Additionally, the infection process itself substantiates the potential activation of one or more of these pathways. For example, within proximity of the SNP lies a glutaredoxin-2 gene. *Glutaredoxin-2* functions to combat oxidative stress (Beer *et al.*, 2004), a probably necessary response following chalkbrood-mediated tissue damage. Within proximity of the SNP also lies the gene mummy (no relation to chalkbrood "mummy"-like appearance). *Mummy* functions during chitin development in tracheal and cuticle structures (Araujo *et al.*, 2005). The low density chitin mesh within the peritrophic membrane (Narayanan, 2004) serves as a filter which may hamper passage of chalkbrood spores. A second chitin biosynthesis pathway member, *chitinase* 3 is located within the interval. As chalkbrood infection occurs through the infiltration of larval mid-gut tissue, variation in the chitin biosynthesis pathway in honey bee larvae may dictate the susceptibility of the gut to infection.

In addition to the above mentioned pathways and their potential involvement, the NF-kappa-B pathway is important for immunity in insects such as in the *Drosophila* response to microbial infection (Lau *et al.*, 2003). The genomic region contains a nuclear factor related to kappa-B binding protein-like 1 as well as an NF-kappa-B repressing factor-like candidate gene that has been shown to modify the transcriptional responses to NF-kappa-B during disease-induced inflammation (Nourbakhsh *et al.*, 2001) functioning as both a repressor and activator of downstream components of the pathway. Sequence variation of the potential candidate NF-kappa-B related genes within our population of honey bee larvae may alter activation of this pathway resulting in differential resistance to chalkbrood.

The simple genetic basis of chalkbrood resistance in larvae suggests that minimal breeding efforts would produce a beneficial degree of control. Analysis of polymorphic markers defining the interval could be used for development of screenable haplotypes. Pre-evaluation of potential breeding queens would ensure that resistance alleles are bred into managed colonies. Considering the success of identifying a genomic region important for chalkbrood resistance via SNP association, fine mapping is underway using additional polymorphic markers (SNPs, microsatellites) within the interval in an attempt to identify the causative gene for resistance. Allelic diversity of the gene within and among the commercial and Russian populations can be analysed to predict the chalkbrood resistance potential already present in current breeding stocks. Depending on the function of the gene(s) responsible for resistance, additional insults such as other fungal or bacterial infections could potentially be mitigated as well. Efforts are already made to breed for hygiene that combats the problem from the perspective of adult workers but the addition of Marker Assisted Selection (MAS) for resistance within the larvae themselves may render chalkbrood a rare or negligible honey bee disease.

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