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Original article

Introgression between *Apis mellifera capensis* Escholtz and *Apis mellifera scutellata* Lepeletier: the sting pheromones

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Summary — The 8 principal components of the alarm pheromone from the stings of guard bees from natural populations of *Apis mellifera capensis* and *A m scutellata* and areas of hybridization were analysed by gas chromatography. *A m scutellata* produces significantly more secretion than *A m capensis*. Autocorrelation analyses of intercolonical variance indicates regions of significant variation in these traits. This introgression coincides with hybridization zones previously defined by other variables. Introgression intensity is related to relative honeybee population density and reveals a probable cyclical bottleneck in 1 area generated by climatic oscillations.

***Apis mellifera capensis* / *Apis mellifera scutellata* / hybridization / sting pheromone**

INTRODUCTION

Coincident, but not concordant, zones of natural hybridization between *Apis mellifera capensis* and *A m scutellata* emerged from analyses of their reproductive biology (Hepburn and Crewe, 1991), morphometry (Crewe *et al,* 1994) and mtDNA haplotypes (Moritz *et al,* 1994). Thus, additional probes were sought to further define the structure of these populations and the intensity and direction of gene flow between them. *Capensis* is extremely docile and *scutellata* exceptionally aggressive, but the constituents of the sting alarm pheromones of their guard bees do not differ (Blum and Fales, 1988).

For other honeybees, the intercolonial variance for these same pheromones was significantly greater in hybrids of landrace crosses (Boch and Rothenbuhler, 1974) or between races of honeybees (Collins *et al,* 1989). Moreover, the constituents of the pheromonal bouquet are highly genetically correlated, their heritabilities approach unity (Collins *et al,* 1987) and gentleness may be dominant over aggressiveness (Kerr *et al,* 1967; Boch and Rothenbuhler, 1974). Thus we measured the variance in alarm pheromone production from the stings of

guard bees on 3 regional transects to assess the introgression of this trait in the natural hybridization process between *capensis* and *scutellata*.

MATERIALS AND METHODS

Worker honeybees were collected from 258 colonies from 29 western, 11 central and 16 eastern localities in the Cape Province. Because *capensis* and *scutellata* are separated on a north–south axis these 3 regions represent the east–west limits and the midpoint of the distribution of these races (fig 1; Hepburn and Crewe, 1991). The distances between localities within each region averaged about 50 km (fig 1). To eliminate age effects, stings from 6 guard bees (assumed to be at least 2 weeks old; Whiffler *et al*, 1988) from each of 4–5 colonies per locality were removed and stored in dichloromethane for gas chromatographic analysis. The samples were measured with a Hewlett–Packard 5890 Series II gas chromatograph fitted with a bonded methyl silicone fused silica capillary column (0.3 mm x 25 m), using *n*-tetradecane as an internal standard. Compounds were quantified with an HP 3396 Series II integrator calibrated against authentic standards of sting pheromone constituents.

Means and standard deviations for each of 8 pheromonal constituents (isopentyl alcohol, *n*-butyl acetate, isopentyl acetate, benzyl alcohol, *n*-octyl acetate, 2-nonanol, benzyl acetate and *n*-decyl acetate) were used to obtain intercolonia variance for each locality. Separate comparisons were then made for locality variances for each of the 3 separate (west, central, and east in figure 1) regions. Finally, localities were grouped in log classes to define areas within each of the 3 main regions to reflect the intensity of variation for each region (*cf*, fig 2). In addition, the complete data set was subjected to a 2-dimensional spatial autocorrelation analysis (Oden and Sokal, 1986) and individual correlograms were obtained for each of the 8 constituents of the sting pheromone. The data were also subjected to a principal compo-

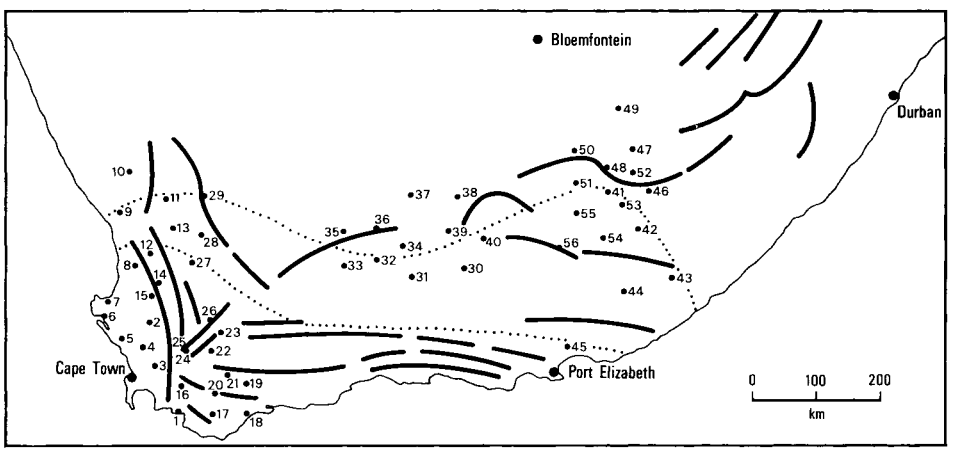

Fig 1. Map of southern Africa showing the localities sampled. Major mountain ranges are indicated as solid stripes. Biologically defined distributions of the 'pure' races are indicated by dotted lines, *capensis* below the lower line and *scutellata* above the upper line, the hybrid zone is the area between the 2 lines (Hepburn and Crewe, 1991). 1. Hermanus, 2. Somerset West, 3. Paarl, 4. Malmesbury, 5. Darling, 6. Langebaan, 7. Laaiplek, 8. Elandsbaai, 9. Lutzville, 10. Bitterfontein, 11. Nieuwoudtville, 12. Clanwilliam, 13. Botterkloof, 14. Citrusdal, 15. Piketberg, 16. Villiersdorp, 17. Napier, 18. Skipskop, 19. Swellendam, 20. Riviersonderend, 21. Bonnievale, 22. Sandvlei, 23. Touwsrivier, 24. Worcester, 25. Ceres, 26. Tweerivier, 27. Elandsvlei, 28. Sonop, 29. Calvinia, 30. Aberdeen, 31. Wiegenaarspoort, 32. Beaufort West, 33. Middelwater, 34. Nelspoort, 35. Rietfontein, 36. Booiskraal, 37. Victoria West, 38. Richmond, 39. Murraysburg, 40. Boesmanskop, 41. Molteno, 42. Queenstown, 43. Stutterheim, 44. Fort Beaufort 45. Addo, 46. Dordrecht, 47. Aliwal North, 48. Burgersdorp, 49. Smithfield, 50. Venterstad, 51. Steynsburg, 52. Jamestown, 53. Sterkstroom, 54. Tarkastad, 55. Hofmeyr, 56. Cradock.

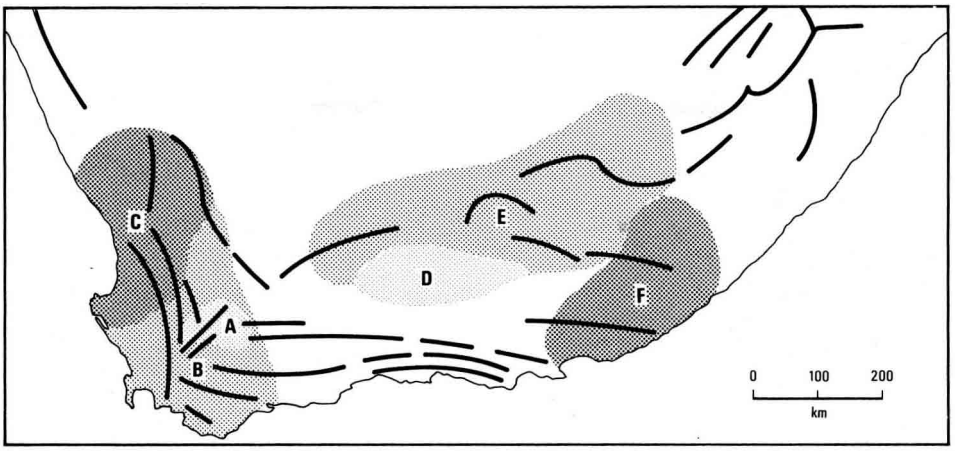

Fig 2. Zones of sting pheromone variance in southern Africa plotted by log-transforming the real variance (table I) and grouping data in 3 log classes: highest variance (0–2) = darkest tone; medium variance (2–4) = medium tone; least variance (4–6) = lightest tone. Letters refer to the groups of localities indicated in table I.

nents analysis to assess whether the total locality data formed a single (1 population) or a group (2 or more populations) of clusters.

Relative honeybee colony population density was estimated indirectly by beekeeper density for each region. There has never been any traditional beekeeping south of the Limpopo River and bees are almost only kept by descendants of immigrants from Europe. Aside from large cities, which are artificial oases, the density of beekeepers reflects the general agricultural carrying capacity of the land. The client data base ($n = 4\ 000$) of the largest national supplier of beekeeping equipment was used to plot beekeeper density per map grid square (about 10 000 km^2). The difficulty of locating beekeepers in each hinterland locality during 8 years of field work provided additional confidence as to the usefulness of the commercial data base. Thus, only relative differences are suggested, not real colonies/km^2, which we cannot yet estimate.

RESULTS

The sting pheromones measured in this study are from honeybees from areas that yield 2 distinct clusters (the races *capensis* and *scutellata*) on morphometric analysis but their hybrids yield a single diffuse cluster (Crewe *et al,* 1994). When the alarm pheromones of the sting were analyzed by principal components analysis only a single cluster was obtained. Thus *capensis* and *scutellata* resolve into a single large, continuous and highly variable population for this trait. Nonetheless, there was a highly significant difference ($P < 0.01$) for the total amount of sting pheromone produced by guard bees from biologically defined areas (fig 1) for pure *capensis* (0.06 μg/bee) and *scutellata* (0.20 μg/bee); the latter produces 3 times more sting pheromone than the former (this data set is not directly relevant to introgression but is available on request).

There were order of magnitude differences of variance in the total pheromonal bouquet as well as for each of the individual constituents between groups of localities (table I). In the 2-dimensional spatial autocorrelation analyses the mean values of the pheromones yielded a north–south inclined

Table I. Intercolonial variances (x 10^3) of sting alarm pheromones for localities shown in figure 1.

Locality and area *	*Isopentyl alcohol*	n-*Butyl acetate*	*Isopentyl acetate*	*Benzyl alcohol*	n-*Octyl acetate*	*2-Nonanol*	*Benzyl acetate*	n-*Decyl acetate*	*Mean variance of 8 pheromones*
1B	0	68	173	0	0	150	42	21	57
2B	0	98	241	0	0	93	31	13	60
3B	0	0	151	0	27	106	44	0	41
4B	0	146	238	0	0	105	50	19	70
5B	199	92	296	0	0	117	48	46	100
6C	1 174	462	571	43	140	459	116	93	382
7C	663	483	594	0	76	728	273	85	363
8C	806	322	285	35	117	294	76	41	247
9C	337	242	100	49	19	89	242	10	136
10C	664	299	337	17	22	101	0	76	190
11C	512	330	1265	28	65	544	187	78	376
12C	665	322	928	6	114	115	0	35	273
13C	188	242	955	0	39	126	0	36	199
14C	399	180	755	12	0	181	0	75	200
15C	117	15	654	6	0	266	0	59	140
16B	231	121	633	8	13	141	55	44	156
17B	14	22	170	71	13	178	174	0	80
18B	242	15	60	60	8	81	52	35	69
19B	166	17	98	24	11	70	32	3	53
20B	157	34	110	100	30	63	174	20	86
21B	70	44	103	13	4	40	22	1	37
22B	52	22	29	18	9	37	9	3	22
23A	36	10	17	18	5	12	10	2	14
24B	83	5	26	29	14	17	24	13	26
25B	0	4	5	0	0	9	0	0	2
26A	27	3	15	9	0	9	9	0	9
27B	143	18	37	35	13	22	84	27	47
28B	425	9	72	26	33	44	65	26	88
29C	352	11	13	2	13	9	9	2	51
30D	78	14	18	0	0	9	0	2	15
31D	78	7	17	9	0	7	5	14	17
32D	68	2	14	19	0	9	10	0	15
33E	90	4	11	36	3	6	82	20	32
34D	72	0	18	13	3	10	0	5	15
35E	4	4	7	19	3	7	382	0	53
36E	66	0	13	37	3	6	117	0	30
37E	152	0	39	17	8	11	15	49	36
38E	29	0	2	20	0	6	102	8	21
39E	351	0	288	96	18	281	342	16	174
40E	151	0	13	19	0	6	96	0	36
41E	808	989	641	28	344	188	144	0	393
42F	2 181	2 002	733	13	539	146	62	0	710
43F	1 573	2 753	829	60	695	234	84	0	779
44F	397	130	537	8	53	94	56	3	160
45F	344	132	348	8	45	138	41	2	132
46E	0	126	179	30	27	255	304	57	122

Table I. Cont.

Locality and area *	*Isopentyl alcohol*	n-*Butyl acetate*	*Isopentyl acetate*	*Benzyl alcohol*	n-*Octyl acetate*	*2-Nonanol*	*Benzyl acetate*	n-*Decyl acetate*	*Mean variance of 8 pheromones*
47E	147	125	235	11	56	122	54	18	96
48E	98	53	153	2	10	61	16	9	50
49E	31	35	257	4	4	124	35	10	63
50E	85	24	68	4	0	69	23	12	36
51E	0	45	99	10	0	80	40	7	35
52E	82	109	138	6	33	109	62	16	69
53E	85	71	145	6	39	174	37	12	71
54E	72	77	159	1	40	95	26	10	60
55E	59	84	172	3	30	138	46	9	68
56E	77	77	188	0	53	77	21	9	63

* Areas are illustrated in figure 2.

plane. This form of analysis does not directly yield map coordinates but gives information on the compass direction of trends in the data. The mean variance values of the pheromones gave a minimum autocorrelation in the central region of the correlogram and maximum values to the north and south (*ie* a saddle, *cf* Oden and Sokal, 1986). Individual pheromone levels and variances for all 8 constituents were significantly autocorrelated.

The geographic distribution of the total variance of the sting pheromones is more readily visualised as areas within regions based on their log-transform values (fig 2) than in the actual correlograms. Introgression intensity is equally high on the west and east sides of the country but is only moderate in the central region. Estimates of probable relative population density for honeybee colonies are given in log-transform classes in figure 3. In general the higher levels of introgression for the sting pheromones occur in those areas of higher relative honeybee population density in the eastern and western regions (compare figs 2 and 3).

DISCUSSION

In the present study we found that the guard bees of *capensis* produced significantly less sting pheromone than those of *scutellata* for groups of guard bees taken from biologically defined areas of the pure races. Similarly, the variances for both groups of pure race samples (within populations) were significantly lower than those for bees identified biologically and morphometrically as hybrids (between populations). Because the heritabilities of the sting pheromones are close to 1 (Collins *et al,* 1987), a large part of the variability assigned to hybrid bees must arise through introgression. This interpretation for natural populations is supported by the heterozygosity obtained from landrace crosses (Boch and Rothenbuhler, 1974) and interracial hybrids (Collins *et al,* 1989).

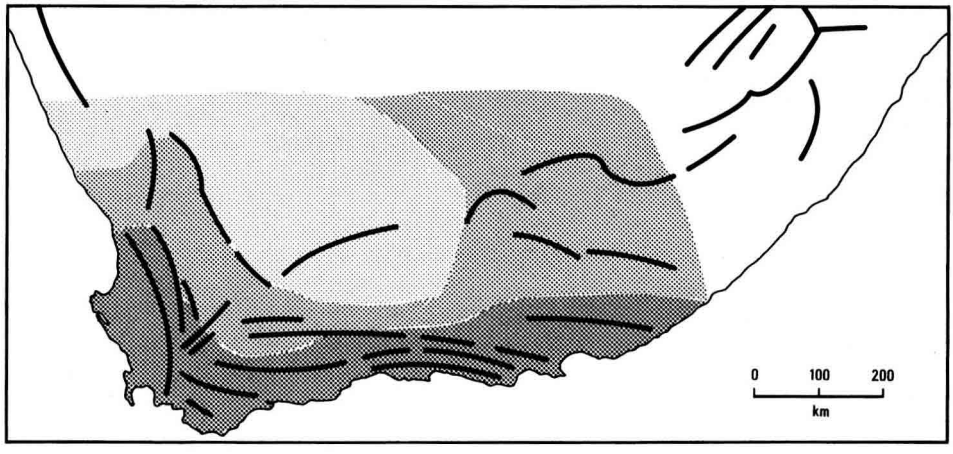

Fig 3. Estimated honeybee colony population density distribution for southern Africa. Zones were plotted by log-transforming known beekeeper density to obtain an index of relative difference. Increasing shade tone density indicates increasing bee colony population density: lightest tone = log classes 0–2; medium tone = log classes 2–4; darkest tone = log classes 4–6.

The dynamics of the introgression for sting pheromones and probable direction of flow can be inferred from a consideration of the introgression of other traits in relation to honeybee population density, topography and climatic cycles. Comparisons of figures 2 and 3 show that the population density of honeybee colonies gradually decreases from south to north for the area considered and so too must the probability of opportunities for gene flow. The direction of gene flow is indicated indirectly by the intensity of variance values spreading in broad heads from *capensis* areas into *scutellata* areas, particularly to the west and east. That the converse is not the dominant direction of flow is supported by other evidence below.

Figure 4 shows the limits of known introgression for several very different *capensis* traits. The mitochondrial DNA haplotype, PoQQa is fixed in *capensis* and occurs with a 100% frequency to the line drawn and extends another 1 000 km north with gradually decreasing frequency (Moritz *et al,* 1994). It may be that mtDNA can introgress further than nuclear DNA because of the *capensis* gene for diploid eggs laid by workers. The limits for the expression of the latter trait are south of the haplotype line as are those obtained from morphometric analyses for hybrids (fig 4). Lastly, continuous qualitative assessments of colony temperament in the field indicated that truly fierce and aggressive bees (a *scutellata* trait) only occur above the northern line limit for the trait 'diploid eggs laid by workers'. Since docility in bees (a *capensis* trait) is probably genetically dominant over aggressiveness (Kerr *et al,* 1967; Boch and Rothenbuhler, 1974) this further suggests gene flow to the north from *capensis* to *scutellata* areas.

Collectively, the data of figure 4 yield broad bands in which a differential introgression of traits between *capensis* and *scutellata* occurs, the dominant (but not the only) direction being from the south. The intensity of introgression is related to apparent honeybee population density and is

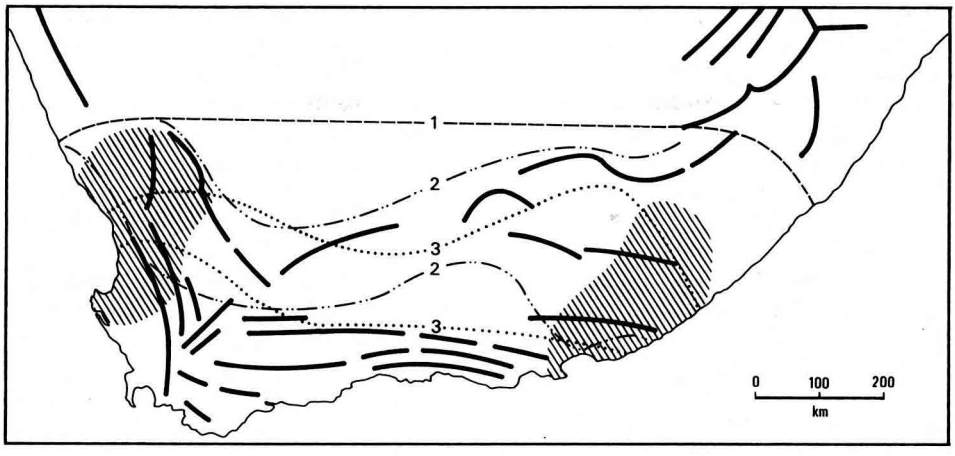

Fig 4. Boundaries for the occurrence of *A m capensis* x *A m scutellata* hybrid traits. Line 1 = northern limit of 100% frequency of the *capensis* haplotype PoQQa (Moritz *et al,* 1994); line 2 = morphometrically defined intermediate zone (Crewe *et al,* 1994); line 3 = area for expression of both haploid and diploid eggs of laying workers (Hepburn and Crewe, 1991); hatched areas to west and east = maximum variance of sting pheromones (fig 2).

sharply defined by the variance values of the sting pheromones. These bands of introgression probably oscillate geographically with alternating decades of severe drought and somewhat wetter years in the Karoo (Tyson, 1986). The reduced population density particularly marked in the central Cape is best interpreted as a classical bottleneck leading to reduced genetic variability in this area.

Superimposed on these cycles are temperate winters to the south (seaside of the mountains) during which *capensis* migrates/absconds; and, more severe weather to the north (great interior highveld) were *scutellata* does not move (Hepburn and Crewe, 1991). *Capensis* also has metabolic, thermoregulatory and reproductive advantages over *scutellata* (Hepburn *et al,* 1993; Hepburn and Allsopp, 1994). In conclusion, the sting pheromone measurements show that this trait is an extremely useful probe for measuring introgression in natural populations of honeybees. Introgression for this trait between *capensis* and *scutellata* reaches maximum intensity within a broad band of other traits previously measured for these 2 races (fig 4).

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Résumé — Introgression entre *Apis mellifera capensis* Escholtz et *Apis mellifera scutellata* Lepeletier : les phéromones d'alarme de l'aiguillon. Les zones d'hybridation entre *A m capensis* et *A m scutellata* coïncident mais ne concordent pas avec les paramètres biologiques et morphologiques. Parce que les phéromones de l'aiguillon présentent une forte héritabilité, nous avons étudié leur production dans 258 colonies réparties en 56 localités d'Afrique du

Sud (fig 1). Nous avons mesuré par chromatographie en phase gazeuse les 8 composés principaux (tableau I) des phéromones de l'aiguillon d'abeilles gardiennes provenant de populations naturelles d'*A m capensis* et d'*A m scutellata* et de populations situées le long de 3 transects d'hybridation. La densité relative des populations d'abeilles a été estimée indirectement d'après la densité des apiculteurs (fig 3). Les écarts types de chaque composé phéromonal ont été additionnés pour obtenir les valeurs de la variance inter-colonies par localité, puis regroupés en zones au sein de chacune des 3 régions (tableau I, figs 1 et 2). Les données ont subi ensuite une analyse d'autocorrélation spatiale à 2 dimensions pour déterminer la direction probable du flux génique et l'intensité des variations entre *A m capensis* et *A m scutellata* pour chacune des régions étudiées (fig 1). Les corrélogrammes résultants ont été visualisés géographiquement par une transformation logarithmique de la variance (fig 2). *A m scutellata* produit 3 fois plus de phéromone de l'aiguillon qu'*A m capensis*. L'hybridation est estimée en termes de variance qui diffère d'un ordre de grandeur d'une population à l'autre. L'introgression a la même intensité dans les régions orientale et occidentale mais est plus faible dans la région centrale (fig 2). Ceci correspond tout à fait avec la densité relative des colonies (fig 3). Ces régions d'introgression intense en ce qui concerne les phéromones de l'aiguillon coïncident avec la répartition du caractère hybride «œufs haploïdes et diploïdes pondus par les ouvrières» et des hybrides définis du point de vue morphologique (fig 4). On en conclut que la position géographique des diverses bandes d'introgression varie selon les décennies en fonction du changement climatique cyclique qui affecte la densité de population.

***A m capensis* / *A m scutellata* / hybridation / phéromone alarme**

Zusammenfassung — Überlappung zwischen *Apis mellifera capensis* Escholtz und *Apis mellifera scutellata* Lepeletier: die Alarmpheromone. Die Zonen der Hybridisierung zwischen *A m capensis* und *A m scutellata* stimmen bezüglich biologischer und morphologischer Parameter nicht überein. Wegen der hohen Heritabilität der Zusammensetzung der Alarmpheromone des Stachels wurde dieses Merkmal bei 258 Völkern an 56 verschiedenen Orten in Süd Afrika untersucht (Abb 1). Die 8 Hauptkomponenten (Tabelle I) der Alarmpheromone der Wächterbienen in natürlichen Populationen von *A m capensis* und *A m scutellata* wurden entlang von 3 Schnittlinien über die Hybridisierungszone gaschromatographisch gemessen. Die relative Dichte der Bienenpopulation wurde indirekt durch die Dichte der Imker bestimmt (Abb 3). Die Standardabweichung für jede Pheromonkomponente wurde zusammengefaßt, um die Varianz der Werte pro Volk und Ort zu bestimmen und danach in Grupenareale für jede der 3 Regionen eingeordnet (Tabelle I, Abb 1,2). Die Daten wurden einer zweidimensionalen räumlichen Autokorrelationsanalyse unterzogen, um die wahrscheinliche Richtung des Genflusses und die Größe der Variation zwischen *A m capensis* und *A m scutellata* für alle 3 untersuchten Regionen zu bestimmen (Abb 1). Die Korrelogramme wurden geographisch dargestellt, indem die Varianzen an den entsprechenden Positionen eingetragen wurden (Abb 2). *A m scutellata* produzierte 3 mal soviel Alarmpheromon wie *A m capensis*. Die Hybridisierung wurde in Form der Varianzen bestimmt, die sich innerhalb der Populationen um eine Grössenordnung unterschieden. Die Stärke der Überlappung war in den westlichen und östlichen Arealen gleich hoch, aber schwächer in der zentralen Kapregion (Abb 2). Diese Verteilung stimmt mit der relativen Populationsdichte der Völker überein (Abb 3). Die Areale mit starker Überlappung der Alarm-

pheromone stimmen mit der Verteilung für das Hybridmerkmal 'haploide und diploide Arbeiterinneneier' und für die morphometrische bestimmten Hybriden überein (Abb 4). Aus diesen Ergebnissen wird geschlossen, daß die geographische Lage der verschiedenen, Überlappungszonen über Jahrzehnte in Abhängigkeit von zyklischen, die Populationsdichte beeinflussenden Änderungen im Klima schwankt.

***A m capensis* / *A m scutellata* / Hybridisierung / Alarmpheromon**

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