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WING VENATION TERATOLOGY IN *APIS MELLIFERA* L. ([1])

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Porporato M., Laurino D., Balzola L., Manino A. – Wing venation teratology in *Apis mellifera* L.

Honey bee (*Apis mellifera* L.) wing venation is quite distinctive and the resulting pattern is currently used in subspecies discrimination. In recent years the forewings of worker bees sampled in *A. m. mellifera*, *A. m. ligustica*, *A. m. carnica*, and hybrid colonies were examined and various abnormalities - due to the presence of both supernumerary and defective veins - were observed. The supernumerary veins were considered as present only if an evident vein length could be detected, while slight thickenings were ignored. Most colonies did not show any teratology in wing venation, while a few ones provided several workers with abnormal wings; in these cases, some individuals showed two or more abnormalities. Spurs of various length protruding from the standard veins were the most frequent abnormalities observed; among them an adventitious distal abscissa of the 2*rs-m* crossvein, a spur protruding from the 2nd abscissa of *Rs* vein into the 1st submarginal cell, and the extension of the *Rs* vein beyond the distal end of the marginal cell were rather common. In some cases two opposed spurs tended to join or a single vein branched, thus defining an open or even closed supernumerary cell. In defective veins the missing stretch varied considerably in length so that in a few cases two contiguous cells merged more or less completely. Wing venation teratology should be taken into account when using the wing venation pattern for a morphometric distinction of honey bee subspecies, and data from abnormal wings should not be acquired, especially if automated procedures are used.

KEY WORDS: forewing, honey bee, morphometry, supernumerary cells, wing veins.

INTRODUCTION

Honey bee (*Apis mellifera* L.) wing venation is quite distinctive, but can be easily referred to the overall pattern shown by the Apoidea (MICHENER, 2000), and more generally by the Hymenoptera. Nomenclature and interpretation of wing veins and of vein bounded cells was much debated during the last two centuries; the relevant literature was recently reviewed by INTOPPA *et al.* (2000) with special reference to Apoidea and a nomenclatural standardisation for bee wings was proposed. Nonetheless various other name systems are still in use to describe bee wing venation (AMIET, 1996; MICHENER, 2000; ENGEL, 2001) and that of ENGEL (2001) was followed in the present work (Fig. I).

Whichever the name given to veins and cells, their presence or absence and their shape are largely used to identify bee taxa at different levels. Particularly, forewing venation morphometry proved to be a useful tool in discriminating *A. mellifera* subspecies (DU PRAW, 1965; RUTTNER *et al.,* 1968, RUTTNER, 1988), strains (STRANGE *et al.,* 2008), and hybrids (RINDERER *et al.,* 1990; NAZZI, 1992; MARLETTO *et al.,* 1994; KELLER *et al.,* 2014). Lately, specific software was developed to speed or fully automate wing data acquisition and analysis under both the standard morphometry or the more innovative geometric morphometrics approach (FLORIS *et al.,* 2002; MEIXNER & MEIXNER, 2004; TOFILSKI, 2004 and 2008; FRANCOY *et al.,* 2008, GERULA *et al.,* 2009; BOUGA *et al.,* 2011).

Honey bee wing venation shows a uniform and regular pattern in most of individual workers, but asymmetries between right and left wing (SMITH *et al.,* 1997) or various teratisms due to the presence of both supernumerary and defective veins are often observed (AKAHIRA & SAKAGAMI, 1959; BÄHRMANN R., 1963; TAN *et al.,* 2008; WĘGRZYNOWICZ, 2010; MAZEED, 2011). These anomalies are often considered as the result of environmental and/or pathogenic troubles during wing development (SOOSE, 1954), but the presence of supernumerary veins is also interpreted as the reacquisition of ancestral traits thus providing an insight into honey bee evolutionary history (TAN *et al.,* 2008).

Since abnormal wing venation was occasionally observed also in Italian bee samples a more extensive investigation was carried out on worker bees from full size colonies and small nuclei in order to understand how widespread wing venation teratology is in *A. mellifera* and to gain some insight into the causes of this phenomenon.

MATERIALS AND METHODS

In the years 2006-2012, samples of 24 worker bees were taken from each of 66 full size colonies; a total of 1,584 worker bees were sampled. Most colonies were of Italian origin, but also two *A. m. mellifera*, three *A. m. carnica*, and one *A. m. macedonica* colonies were sampled. In 2012, 99 newly mated queens were obtained from 20 Italian queen breeders (five queens from each breeder except a single one who provided four queens only) for a survey on

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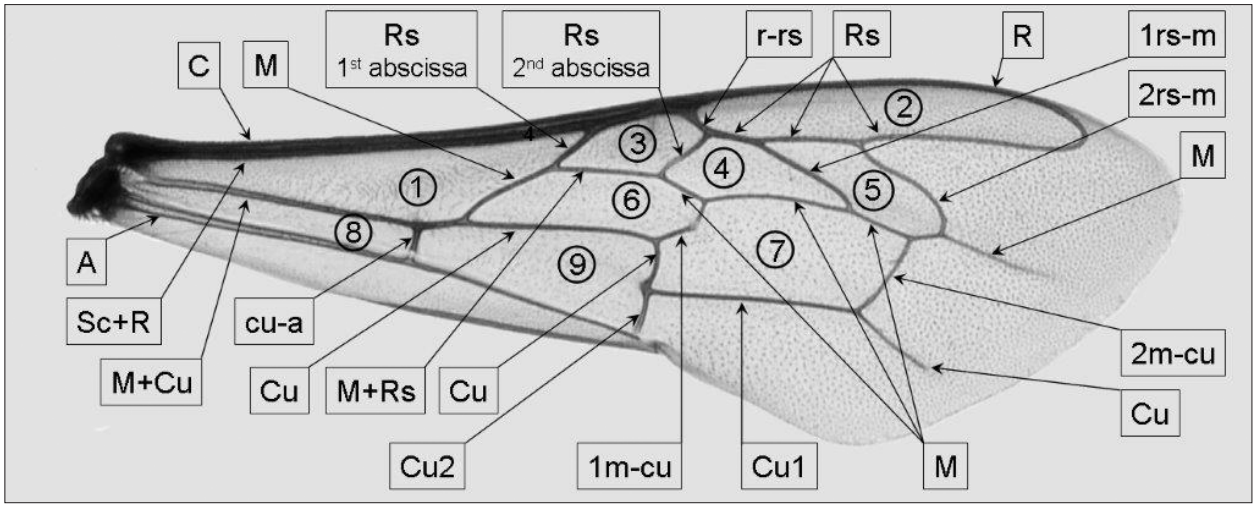


Fig. I – Right mesothoracic *Apis mellifera* wing showing a regular venation with cells defined by longitudinal veins and transverse crossveins; the latters are denoted by two letters indicating which longitudinal veins they connect (anterior vein first, posterior second) and abbreviated by lowercase letters. 1: radial cell; 2: marginal cell; 3: 1st submarginal cell; 4: 2nd submarginal cell; 5: 3rd submarginal cell, 6: 1st medial cell; 7: 2nd medial cell; 8: 1st cubital cell; 9: 2nd cubital cell. *A*: anal vein; *C*: costal vein; *Cu*: cubital vein; *M*: medial (basal) vein; *R*: radial vein; *Rs*: radial sector; *Sc*: subcostal vein. The costal cell between *C* and *Sc+M* veins is so reduced to appear virtually absent.

the occurrence of honey bee queen anomalies and diseases; each queen was sent in a queen cage with some attending worker bees, taken from the queen mating nucleus; in all, 746 individuals were collected (37.3 ± 8.66 worker bees from each queen breeder). Worker bees sampled in a full size colony with a long established queen are reasonably daughters of the colony queen, while adult workers in a professional queen breeder mating nucleus are at any moment most likely daughters, not of the newly mated queen present in the nucleus, but of previous queens or came from other colonies present in the mating yard; therefore attending workers from queen cages can be regarded at best as a random sample of the breeder's stock. From this point of view, nine of the breeders who provided the queens were on the official Italian register of queen breeders and their stocks were certified as pure *A. m. ligustica* strains.

The right forewing of each worker bee was taken off the thorax, placed between glasses in a slide frame and scanned at 3,200 dpi with an Epson Perfection 4490 Photo scanner. The resulting images were enlarged and observed to trace wing venation anomalies; supernumerary veins were considered as present only if an evident vein length could be detected, while slight thickenings were ignored.

RESULTS

Most of the worker bees sampled from full size colonies showed a regular wing venation, but in 69 wings (4.36% of the total) various teratisms were observed (Tab. 1); no abnormal wings were found in 26 samples and a single abnormal wing was present in other 26 samples, while up to five abnormal wings were observed in the remaining samples. Some wings showed two or more teratisms so that a total of 85 wing venation abnormalities were observed. On the contrary, significantly more teratisms (χ^2 = 74.100 with 1 degree of freedom; P = <0.001) were present in worker bees sampled from mating nuclei: 119 defective wings with a total of 130 wing venation abnormalities (Tab. 1). Moreover only the accompanying workers from a single queen breeder showed no abnormal wings and no statistically significant difference in teratism occurrence was observed between the samples coming from facilities enrolled on the official Italian register of queen breeders and the others (χ^2 = 0.419 with 1 degree of freedom; P = 0.518).

Some wings showed the standard venation pattern, but an irregular course of one, most often the *1rs-m* crossvein, or more veins was observed (Fig. II, a).

Spurs of various length protruding from the standard veins were the most frequent abnormalities observed. Among them, an adventitious distal abscissa of the *2rs-m* crossvein (Fig. II, d), a spur protruding from the 2nd abscissa of the *Rs* vein into the 1st submarginal cell (Fig. II, c), and the extension of the *Rs* vein beyond the distal end of the marginal cell (Fig. II, b) were rather common.

In some cases two opposed spurs tended to join (Fig. II, e) or a single vein, most often the *1rs-m* crossvein, branched, thus defining an open (Fig. III, a and b) or even closed (Fig. III, b) supernumerary cell.

The presence of defective veins was less common than the above mentioned abnormalities; in this instance, the missing stretch of the defective vein varied considerably in length so that in a few cases two contiguous cells merged more or less completely (Fig. IV, a, b, c, d, e).

DISCUSSION

The incidence of wing venation anomalies places the observed samples - taking into account the different approaches adopted by the authors who investigated such a teratology (AKAHIRA & SAKAGAMI, 1959; BÄHRMANN R., 1963; TAN *et al.,* 2008; WĘGRZYNOWICZ *et al.,* 2010; MAZEED, 2011) - roughly at the same levels reported in the literature. Nevertheless the remarkable differences observed between worker bees from full size colonies and those from mating nuclei cannot be overlooked. Most probably worker bees reared in mating nuclei are more exposed to temperature fluctuations during metamorphosis than those reared in full size colonies (SOOSE,

Table 1 – Teratology of honey bee worker forewing observed in full size colonies and mating nuclei.

Teratology	colonies		mating nuclei	
	n.	%	n.	%
irregular vein course	**5**	**5.88**	**7**	**5.38**
supernumerary veins	**57**	**67.06**	**98**	**75.38**
adventitious distal abscissa of the *2rs-m* crossvein	37	43.53	53	40.77
extension of the *Rs* vein from the distal end of the marginal cell	5	5.88	20	15.38
spur protruding from the 2nd abscissa of the *Rs* vein into the 1st submarginal cell	11	12.94	14	10.77
spur protruding from *1rs-m* at the forking place	2	2.35	8	6.15
other spurs	2	2.35	3	2.31
supernumerary cells	**12**	**14.12**	**18**	**13.85**
supernumerary submarginal cell	9	10.59	7	5.38
forked *1rs-m* crossvein forming an incomplete supernumerary submarginal cell	1	1.18	11	8.46
other incomplete supernumerary cells	2	2.35	-	-
defective veins	**11**	**12.94**	**7**	**5.38**
incomplete *1rs-m* crossvein causing a partial merging of 2nd and 3rd submarginal cells	6	7.06	7	5.38
incomplete *1m-cu* crossvein causing a partial merging of 1st and 2nd medial cells	2	2.35	-	-
other defective veins	3	3.53	-	-
total	**85**	**100.00**	**130**	**100.00**

1954), but also the possibility that queen breeder bee stocks showed some degree of inbreeding should be considered.

The adventitious distal abscissa of the *2rs-m* crossvein so often observed is clearly the same wing venation anomaly reported by TAN *et al.* (2008) and one could fully agree with their conclusions on the phenomenon. Also the extension of the *Rs* vein beyond the distal end of the marginal cell could be regarded as the reappearance of a vein present in other bee genera (like *Melitturga* for instance) and lost by *Apis* during its evolution.

As observed long ago by ALPATOV (1929), various teratisms affected the *1rs-m* crossvein also in the examined samples thus suggesting that this vein is a rather instable one. On the other hand, the number of submarginal cells in the bee forewing is a widely used feature to distinguish among bee genera, though there is not agreement whether the 2nd abscissa of *Rs* or the *1rs-m* are lacking when only two submarginal cells are present and either case may be true for different species (TOFILSKI, 2011).

Since the wing venation pattern is widely used for the distinction of honey bee subspecies with a morphometric approach, any venation teratology observed in the worker bee sample used should not be overlooked because it could lead to a wrong attribution of the sample. In any case, data from abnormal wings should not be acquired and the whole sample should be prudentially discarded if two or more wings show any abnormality especially if automated procedures are used for data retrieving. Moreover worker bee samples should be obtained from full size colonies and not from small nuclei or from queen cages since these have proved to be more prone to show wing venation teratisms.

REFERENCES

AKAHIRA Y., SAKAGAMI S.F., 1959 – *Observations on the Variability of Wing Venation in the Honeybees*. - J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool., 14: 175-184.

ALPATOV W.W., 1929 – *Biometrical studies on variation and races of the honeybee* Apis mellifera *L.* - Q. Rev. Biol., 4(1): 1-58.

AMIET F., 1996. – *Hymenoptera Apidae, 1. Teil. Allgemeiner Teil, Gattungsschlüssel, die Gattungen* Apis, Bombus *und* Psithyrus. - Insecta Helvetica 12, Musée d'Histoire naturelle, Neuchâtel, 98 pp.

BÄHRMANN R., 1963 – *Zum Vorkommen von Anomalien im Flügelgeäder der Honigbiene.* - Arch. Bienenkunde, 40: 49-58.

BOUGA M., ALAUX C., BIENKOWSKA M., BÜCHLER R., CARRECK N.L., CAUIA E., CHLEBO R., DAHLE B., DALL'OLIO R., DE LA RÚA P., GREGORC A., IVANOVA E., KENCE A., KENCE M., KEZIC N., KIPRIJANOVSKA H., KOZMUS P., KRYGER P., LE CONTE Y., LODESANI M., MURILHAS A.M., SICEANU A., SOLAND G., ALEKSANDAR UZUNOV A., WILDE J., 2011 – *A review of methods for discrimination of honey bee populations as applied to European beekeeping.* - J. apic. Res., 50: 51-84.

DU PRAW E.J., 1965 – *The recognition and handling of honeybee specimens in non-Linnean taxonomy.* - J. apic. Res., 4: 71-84.

ENGEL M.S., 2001 – *A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera).* - Bull. Am. Mus. Nat. Hist., 259, 192 pp.

FLORIS I., SATTA A., RUIU L., 2002 – *Un database per Api e Mieli della Sardegna*. - Regione autonoma della Sardegna, Cagliari, 66 pp.

FRANCOY T.M., WITTMANN D., DRAUSCHKE M., MÜLLER S., STEINHAGE V., BEZERRA-LAURE M.A.F., DE JONG D., GONÇALVES, L.S., 2008 – *Identification of Africanized honey bees through wing morphometrics: Two fast and efficient procedures.* - Apidologie, 39: 488-494.

GERULA D., TOFILSKI A., WĘGRZYNOWICZ P., SKOWRONEK W., 2009 – *Computer-assisted discrimination of honeybee subspecies used for breeding in Poland.* - J. apic. Sci., 53: 105-114.

KELLER, E.M., HARRIS, I., CROSS, P. 2014 – *Identifying suitable queen rearing sites of* Apis mellifera mellifera *at a regional scale using morphometrics*. - J. apic. Res., 53: 279-287.

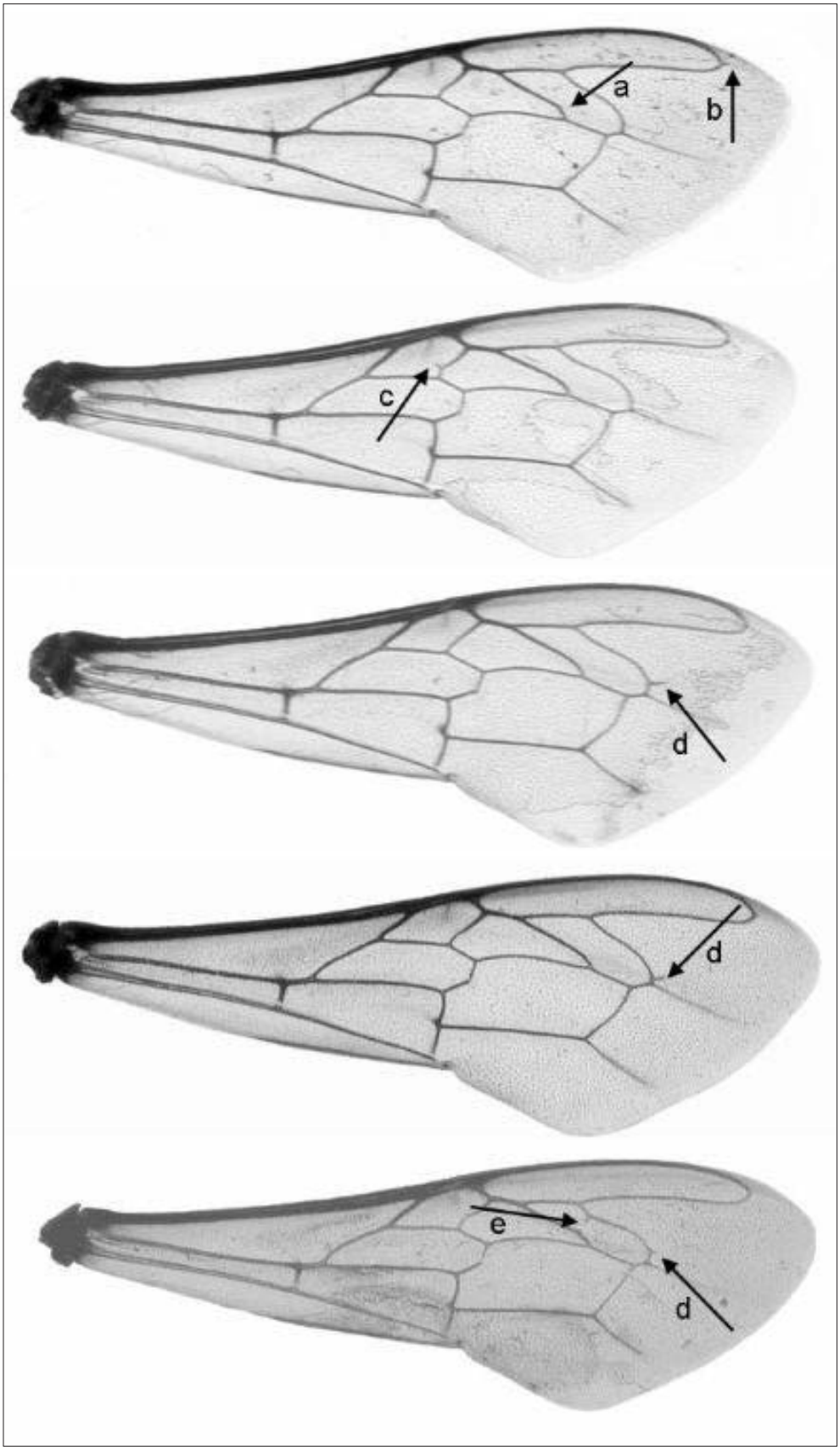
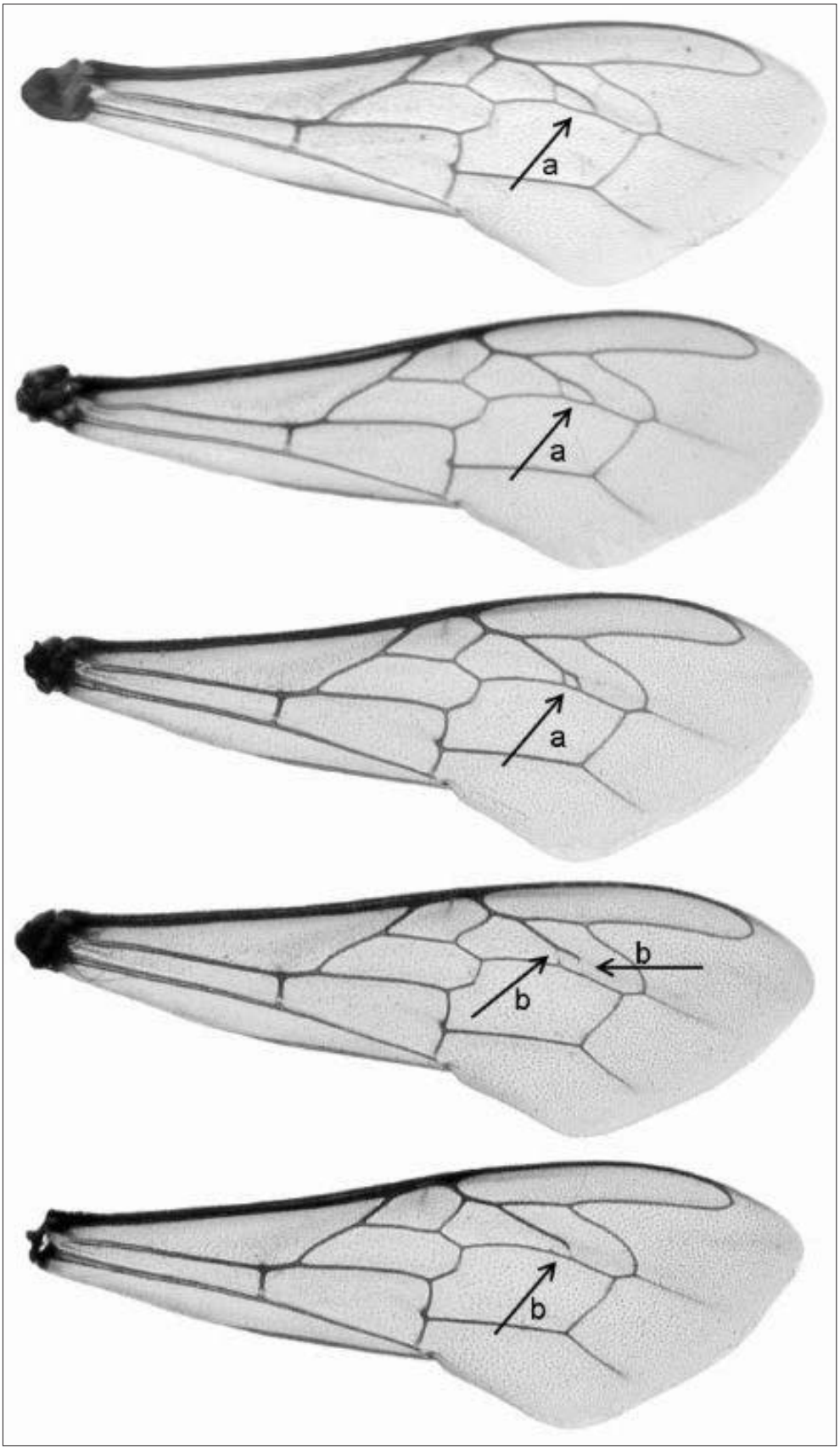


Fig. II – Right mesothoracic *Apis mellifera* wings showing an irregular course of the *1rs-m* crossvein (a) and several spurs protruding from various veins: abscissa of *Rs* beyond the distal end of the marginal cell (b); spur protruding from the 2nd abscissa of the Rs vein into the 1st submarginal cell (c); adventitious distal abscissa of the *2rs-m* crossvein (d); two spurs protruding from *1rs-m* and *2rs-m* crossveins, spurs which nearly meet dividing the 3rd submarginal cell into two separate cells (e).



Fig, III – Right mesothoracic *Apis mellifera* wings showing a forked *1rs-m* crossvein: a) complete vein forming a closed supernumerary submarginal cell; b) incomplete, but forked vein forming an open supernumerary submarginal cell.

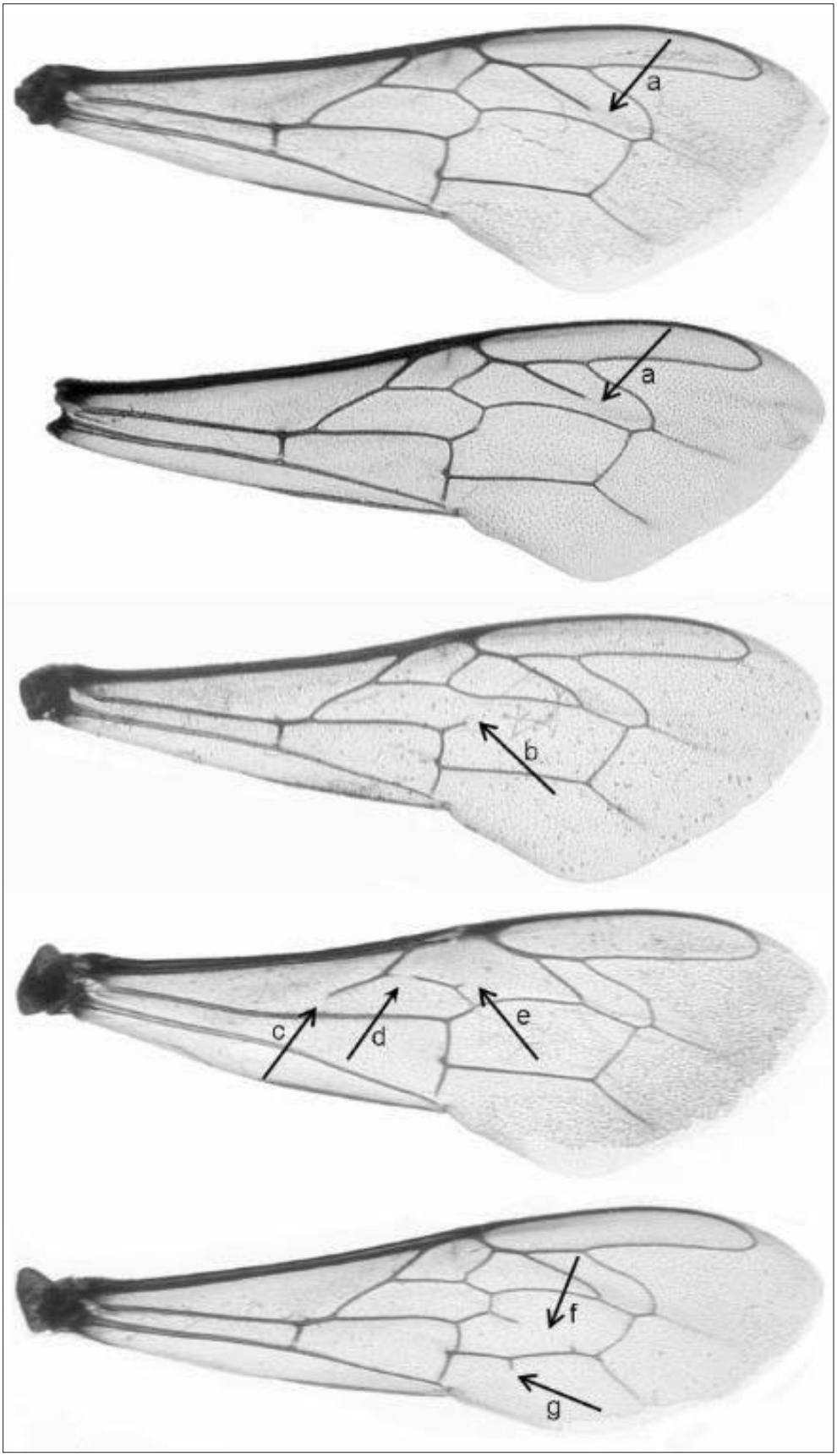


Fig. IV – Right mesothoracic *Apis mellifera* wings showing: a) incomplete *1rs-m* crossvein causing a partial merging of 2nd and 3rd submarginal cells; b) incomplete *1m-cu* crossvein causing a partial merging of 1st and 2nd medial cells; c) *M* and d) *Rs+M* veins interrupted, e) 2nd abscissa of the *Rs* vein reduced to a spur so that 1st and 2nd submarginal cells merge; f) incomplete supernumerary vein dividing the 2nd medial cell; g) spur protruding from the *Cu1* vein.

INTOPPA F., PIAZZA M.G., BOLCHI SERINI G., 2000 – *Nomenclatura e interpretazione della venulazione alare degli Apoidei: una revisione critica*.- Redia, 83, Appendice: 1-24.

MAZEED A.M.M., 2011 – *Anomalies and asymmetry of wing venation pattern in Carniolan and Egyptian bee populations in Egypt*. - Egypt. Acad. J. Biolog. Sci., 4 (1): 149- 161.

MARLETTO F., MANINO A., BALBONI G., 1994 – *Indagini biometriche su popolazioni di* Apis mellifera *L. delle Alpi occidentali.* - Apicolt. mod., 75: 213-223.

MEIXNER A., MEIXNER M.D., 2004 – *Bee2 morphometric software, 1996–2004*. Frankfurt, Germany.

MICHENER C.D., 2000 – *The Bees of the World*. The Johns Hopkins University Press, Baltimore, Maryland, XIV+913 pp.

NAZZI F., 1992 – *Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy.* - Apidologie 23: 89-96.

RINDERER T.T., DALY H.V., SYLVESTER H.A., COLLINS A.M., BUCO S.M., HELLMICH R.L., DANKA R.G., 1990 – *Morphometric differences among Africanized and European honey bees and their F_1 hybrids (Hymenoptera: Apidae).* - Ann. Ent. Soc. America, 83: 346-351.

RUTTNER F., 1988 – *Biogeography and taxonomy of honeybees*. Springer-Verlag, Berlin, XII+284 pp.

RUTTNER F, TASSENCOURT L, LOUVEAUX J., 1968 – *Biometrical-statistical analysis of the geographic variability of* Apis mellifera *L. 1. Materials and Methods*. – Apidologie, 9: 363-381.

SMITH D.R., CRESPI B.J., BOOKSTEIN F.L., 1997 – *Fluctuating asymmetry in the honey bee,* Apis mellifera*: effects of ploidy and hybridization*. - J. evol. Biol., 10: 551 574:

SOOSE E., 1954 – *Einfluss der Temperatur auf die Ausgestaltung Flügelindex und Panzerfarbe der Honigbiene* (Apis mell*.).* - Arch. Bienenkunde, 31: 49-66.

STRANGE J.P., GARNERY L., SHEPPARD W.S., 2008 – *Morphological and molecular characterization of the Landes honey bee (*Apis mellifera *L.) ecotype for genetic conservation*. – J. Insect Conserv., 12: 527–537.

TAN K., FUCHS S., ENGEL M.S., 2008 – *An adventitious distal abscissa in the forewing of honey bees (Hymenoptera: Apidae*: Apis). – Apidologie, 39: 674-682.

TOFILSKI A., 2004 – *DrawWing, a program for numerical description of insect wings*. - J. Insect Sci., 4: 17-22.

TOFILSKI A., 2008 – *Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies.* - Apidologie, 39: 558-563.

TOFILSKI A., 2011 – *Homology of submarginal crossveinsin forewing of bees*. - J. apic. Sci., 55: 131-140.

WĘGRZYNOWICZ P., GERULA D., PANASIUK B., BIEŃKOWSKA M., 2010 – *Anomalies in wings of* Apis mellifera. http://www.inhort.pl/files/program_wieloletni/wykaz_publikacji/obszar6/Anomalia%20skrzydel_Anomalies%20in%20wings%20of%20Apis%20mellifera.pdf