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T. Balenghien, F. Fouque, Philippe Sabatier, D. Bicout. Horse-, bird-, and human-seeking behavior and seasonal abundance of mosquitoes in a West Nile virus focus of southern France.. Journal of Medical Entomology, 2006, 43 (5), pp.936-46. 10.1093/jmedent/43.5.936 . pasteur-00308950

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Horse-, Bird-, and Human-Seeking Behavior and Seasonal Abundance of Mosquitoes in a West Nile Virus Focus of Southern France

T. BALENGHIEN,[1] F. FOUQUE,[2] P. SABATIER,[1] AND D. J. BICOUT[1]

J. Med. Entomol. 43(5): 936–946 (2006)

ABSTRACT After 35 yr of disease absence, West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) circulation has been regularly detected in the Camargue region (southern France) since 2000. WNV was isolated from *Culex modestus* Ficalbi, which was considered the main vector in southern France after horse outbreaks in the 1960s. Recent WNV transmissions outside of the *Cx. modestus* distribution suggested the existence of other vectors. To study potential WNV vectors, horse- and bird-baited traps and human landing collections of mosquitoes were carried out weekly from May to October 2004 at two Camargue sites: one site in a wet area and the other site in a dry area, both chosen for their past history of WNV transmission. At the wet site, the most abundant species in bird-baited traps were *Culex pipiens* L. and *Cx. modestus*; both species also were found in lower proportions on horses and humans. The most abundant species in horse-baited traps and human landing collections were *Aedes caspius* (Pallas), *Aedes vexans* (Meigen), and *Anopheles hyrcanus* (Pallas) sensu lato; some of these species were occasionally collected with avian blood at the end of the summer. *Anopheles maculipennis* Meigen sensu lato was an abundant horse feeder, but it was rarely collected landing on human bait and never contained avian blood. At the dry site, *Cx. pipiens* was the most abundant species in bird- and horse-baited traps. The seasonal and circadian dynamics of these species are analyzed, and their potential in WNV transmission in Camargue discussed.

KEY WORDS Camargue, host-baited collections, mosquito potential vectors, West Nile fever

West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) was first isolated in 1937 from the blood of a febrile woman in Uganda (Smithburn et al. 1940), and its transmission by mosquitoes was experimentally established in 1943 (Philip and Smadel 1943). This arbovirus was reported to cause mild disease and was reisolated from apparently healthy children in Egypt in 1950 (Melnick et al. 1951). During the 1950s, WNV was suspected to be responsible for human disease outbreaks in Israel (Bernkopf et al. 1953), and its etiological role in human encephalitis was established (Southam and Moore 1954). At the same time, ecological studies undertaken in Sindbis district in Egypt described WNV transmission cycles, involving mosquitoes (mainly *Culex* species) as vectors, birds as amplifying hosts, and humans and horses as sensitive but probably dead-end hosts (Taylor et al. 1956). During the subsequent decades, WNV was isolated in many countries of Africa, the Middle East, Asia, and Europe (Murgue et al. 2002), and its transmission was studied after outbreaks in Israel (Goldblum et al. 1954), France (Panthier 1968), and South-Africa (McIntosh et al. 1967).

During the past 10 yr, severe WNV outbreaks occurred around the Mediterranean basin, with human mortality in Algeria in 1994 (Le Guenno et al. 1996), Romania in 1996 (Savage et al. 1999), Tunisia in 1997 (Triki et al. 2001), Russia in 1999 (Platonov et al. 2001), and Israel in 2000 (Weinberger et al. 2001). Horse epizootics also were reported from Morocco in 1996 (Tber Abdelhaq 1996), Italy in 1998 (Autorino et al. 2002), France in 2000 (Murgue et al. 2001), and Morocco again in 2003 (Schuffenecker et al. 2005). In 1999, two fatal human cases of West Nile fever were reported from Israel (Giladi et al. 2001), after WNV mortality in bird populations the previous year (Malkinson et al. 2002). The same year, the emergence of WNV was reported for the first time from North America associated with the death of seven people, several horses, and thousands of wild birds in New York city (Garmendia et al. 2001). The WNV strain isolated in The United States was closely related to a strain isolated from a goose in Israel in 1998 (Lanciotti et al. 1999), suggesting an introduction of the virus from Israel. After this first outbreak, WNV became a major problem for public health in the United States (Hayes et al. 2005) with ≈20,000 human infections and ≈800 fatal cases (CDC statistics, http://www.cdc.gov/ncidod/dvbid/westnile/) and the virus spread to Canada (Gancz et al. 2004), Mexico (Estrada-Franco et al. 2003), and the Caribbean islands (Dupuis et al. 2003, Komar et al. 2003, Quirin et al. 2004).

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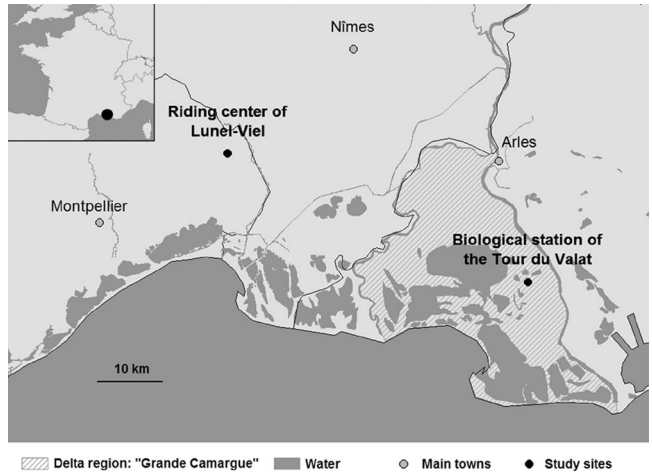


Fig. 1. Location of the study sites: the biological station of the Tour du Valat and the riding center of Lunel-Viel.

In Camargue (region of the Rhône River delta in southern France), summer influenza-like syndromes in humans, and neurological syndromes in horses called "lourdige" were described since the beginning of the 20th century (Panthier et al. 1968, Joubert et al. 1970). In 1962, a large number of encephalitis syndromes occurred in horses associated with report of human cases in Camargue (Panthier 1968). Epidemiological studies in the subsequent years confirmed the implication of WNV, which was isolated in 1964 from field-collected mosquito pools of *Culex modestus* Ficalbi and from the blood of two entomologists (Hannoun et al. 1964), and, in 1965, from the brain of a foal with encephalitis (Panthier et al. 1966). Further serological investigations suggest the existence of a primary WNV focus in Camargue, a smaller focus around the city of Cannes, and some serological evidence from Corsica (Joubert 1975). The entomological studies carried out in the 1960s considered *Cx. modestus*, a species breeding in reed marshes or rice fields and aggressively feeding on birds, humans, and other mammals, as the main WNV vector. However, these authors did not exclude the role of *Culex pipiens* L. as an enzootic vector (Mouchet et al. 1970). After the first WNV outbreak, the virus seemed to persist in the environment as detected by seroconversion in rabbits in 1978 (Rollin et al. 1982) but failed to produce human or equine cases. Investigations in a large population of wild rabbits in 1986 showed a very low level of WNV antibodies (Le Lay-Rogues et al. 1990). More recently, and like other European countries, France faced new WNV episodes. A large equine outbreak occurred in 2000 in western Camargue (Durand et al. 2002), and serological evidence of virus circulation was reported in the bird surveillance network in 2001 and 2002 (Zeller and Schuffenecker 2004). In 2003, five human cases and an important WNV circulation in horse populations were reported (Del Giudice et al. 2004, Durand et al. 2005) from the Var department (Côte d'Azur region). Recent WNV transmission in dry areas inappropriate for development of *Cx. modestus* suggests transmission cycles involving other vectors.

In this context, a research program was undertaken to study potential WNV vectors and improve knowledge of WNV transmission cycles in southern France. To be implicated as a vector, a mosquito species must satisfy specific requirements: a biology compatible with the required host–vector contact (e.g., adequate host preferences, activity during the period of virus circulation), a demonstrated laboratory vector competence, and virus isolation from field-collected individuals (Turell et al. 2002). To explore the first requirement, weekly host-baited collections were carried out from May to October 2004 by using bird- and horse-baited traps and human landing collections. Collections were performed in one wet and one dry site, both with past evidence of WNV transmission. Mosquito diversity and abundance were analyzed and compared for these two sites. The population dynamics and circadian biting activities of the most important species are discussed as well as the potential role of vectors based on host preference and relative abundance.

Materials and Methods

Collection Sites and Associated Environment. The Camargue region includes the Rhône River delta (called "Grande Camargue") and western areas of the delta, with southern wet areas and northern dry areas (Fig. 1). The wet areas of Camargue host large populations of migratory and resident birds and extensive breeding of cattle and horses. Human and horse WNV outbreaks occur both in wet areas and in bordering dryer areas. Study sites were therefore chosen in these two contrasted zones. One site was located at the biological station of the Tour du Valat in the delta area, and the other site was located in a riding center at Lunel-Viel in a bordering dry area (Fig. 1).

The ecological mosaic of the Rhône River delta results from the influence of the sea in the south and



Fig. 2. Mosquito collection methods: horse- and bird-baited traps and human landing.

agricultural activities in the north (Devaux 1978), leading to a south-north gradient of salinity (originating from the mixing of the tide water from the sea and the irrigation system of fresh water from the Rhône River). Localized in the center of the delta and east of the Vaccarès pond, the domain of the Tour du Valat includes a nature reserve (Hoffmann et al. 1968). The southern landscapes are under the influence of the saltwater and exclusively composed of associations of halophytic plants (locally called "sansouire": *Arthrocnemum glaucum* Ungern-Sternberg, *Arthrocnemum perenne* (Miller), and *Salicornia herbacea* L.) and salt ponds (*Zostera noltii* (Horneman) and *Ruppia cirrhosa* [Petagna]). The network of southern salt ponds is connected with the sea during the winter, when pond levels are high, or during storm events. The tides are very low for the Mediterranean Sea (mean of 40 cm). The vegetation associated with fresh water is composed of reed marshes (*Phragmites australis* (Trin.), *Potamogeton pectinatus* L., and *Myriophyllum spicatum* L.), wet meadows (*Juncus maritimus* Lam., *Trifolium* species, and *Medicago* species), and riverine forest (*Populus alba* L., *Populus nigra* L., *Fraxinus angustifolia* Vahl, *Quercus pubescens* Willd., *Laurus nobilis* L., and *Tamarix gallica* L.). Rice fields and pastures are the third ecological complex of this area. In the delta, flood levels are conditioned by rainfall and artificial flooding through a complex network of canals maintained for human activities (cultivation, livestock productions, and hunting and fishing), colonized by reeds, and bordered by trees (*Tamarix gallica* and *Eleagnus angustifolia* L.). The extensive breeding of Camargue bulls in large marshes is an important part of agricultural activities in Camargue. The Camargue horse is essential for the "gardians" (local cow shepherds) to sort out herds of bulls and is one of the major folk symbols of Camargue.

In contrast, the Lunel-Viel village is situated in a dry area of the Camargue region (Dupias et al. 1966), where agricultural areas are composed mainly of vineyards, forage production, and fallow fields surrounded by Mediterranean vegetation (*Quercus ilex* L., *Pinus halepensis* Miller, and *Quercus pubescens*). The study site was located in a riding center near a small river, partially drained at the end of the summer, and bordered by poplars (*P. alba*).

The sites under study were selected according to epidemiological considerations. After the WNV episode of 1962 (Panthier et al. 1968, Joubert et al. 1970), entomological WNV studies in the 1960s were carried out in the Tour du Valat area (Mouchet et al. 1970). The riding center of Lunel-Viel was chosen because a clinical infection in a resident horse was reported during the 2000 outbreak (Durand et al. 2002).

Meteorological Data. Temperature and rainfall data were obtained from the meteorological stations (Météo France) in the Tour du Valat domain and in the town of Vérargues, situated at ≈3 km from the site of Lunel-Viel.

Mosquito Collections. Mosquito collections were carried out weekly from 10 May to 11 October 2004 at the Tour du Valat site and from 27 May to 14 October 2004 at the Lunel-Viel site by using horse- and bird-baited traps and human landing collections (Fig. 2). The animals used in baited traps were the same during all collections. We used an 11-yr-old Camargue gelding at the Tour du Valat site and a 14-yr-old "selle française" mare at the Lunel-Viel site. We used two 1-yr-old ducks of the "Mulard" breed at the Tour du Valat site and two 3-yr-old male ducks of the "Barbarie" breed at the Lunel-Viel site. Outside of the collection periods, the animals were maintained by their owners.

Horse-baited traps were net boxes (4 by 4 by 3 m), with a free space of ≈10 cm from the ground allowing mosquitoes to enter. The bird-baited traps were net and wood cages (0.5 by 0.5 by 0.7 m) with a gutter-shaped opening and a mosquito-collecting chamber at the top of the cage. The horse-baited traps were installed in an open area inside a forested area at the Tour du Valat site and in a pasture near the river at the Lunel-Viel site. At each site, two bird-baited traps with one duck per trap were suspended in a tree ≈1 and ≈8 m above the ground. Animal-baited traps were installed at noon and operated for 24 h. Mosquitoes were collected every 4 h (at 4, 8, 12, 16, 20, and 24 h) with a backpack aspirator (Modified CDC Backpack Aspirator model 1412, John W. Hock, Gainesville, FL)

in the horse-baited traps and with a hand aspirator (Aspirator, model 1135A, BioQuip, Gardena, CA) in the bird-baited traps. Mosquitoes landing on humans were collected with a hand aspirator by two collectors exposing their calves for 15 min every 4 h for 24 h starting at noon.

Collected mosquitoes were sorted by species (identified using morphological keys, Schaffner et al. 2001), by sex, by whether they were engorged or unengorged, and counted. Mosquitoes were frozen and stored at −20°C for further investigations.

Bloodmeal analysis was carried out on engorged female *Aedes*, *Anopheles*, and *Coquillettidia* collected in bird-baited traps to confirm the ability of these species to feed on bird. A direct enzyme-linked immunosorbent assay (ELISA) was used for bloodmeal identification, following the method described by Beier et al. (1988). All bloodmeals were tested with antisera (Sigma-Aldrich, Lyon, France) against human, bird, horse, and cattle, with positive and negative controls.

Results

Meteorological Data. In 2004, total rainfall was 476 mm at the Tour du Valat site and 585 mm at the Lunel-Viel site. During the collection period, rainfall was sporadic from May to July at both sites (Fig. 3). Total rainfall in August, September, and October was 8, 72, and 157 mm at the Tour du Valat site and 71, 88, and 92 mm at the Lunel-Viel site. In these areas, total annual rainfall usually ranges between 400 and 700 mm and is irregular, with important storms mainly at the end of summer (with up to 200 ml during a day).

The mean daily minimum and maximum temperatures were, at the Tour du Valat site, 10.8 and 20.4°C during the spring, 17.4 and 28.0°C during the summer, and 9.1 and 16.5°C during the fall and at the Lunel-Viel site, 9.5 and 22.4°C during the spring, 16.2 and 30.2°C during the summer, and 7.6 and 17.6°C during the fall. These values reflect a Mediterranean climate, characterized by warm and dry summer and mild and wet winter. In southern France, mean temperatures usually range between 0 and 10°C during winter and between 15 and 30°C during summer. The weekly temperature means were similar at both sites, but the minimum and the maximum temperature range was smaller at the Tour du Valat site, because of the influence of the sea. The Mediterranean Sea surface temperature lies between 10 and 15°C during the winter and 21 and 30°C during the summer.

Diversity. In total, 141,071 mosquitoes of 14 different species were collected (22 collection days) at the Tour du Valat site and 1,646 mosquitoes of nine species (21 collection days) at the Lunel-Viel site (Table 1).

At the Tour du Valat site, *Aedes caspius* (Pallas), *Aedes vexans* (Meigen), *Anopheles maculipennis* Meigen sensu lato, and to a lesser extent *Anopheles hyrcanus* (Pallas) sensu lato represented 98.8% of engorged females collected on the horse. The same species, except for *An. maculipennis*, represented 95.5% of females collected landing on human bait. *Cx. pipiens* and *Cx. modestus* represented 97.3% of engorged females collected on birds. *Cx. modestus* fed mainly at ground level, and *Cx. pipiens* was found equally at ground and canopy levels.

At the Lunel-Viel site *Cx. pipiens*, *Ae. caspius*, and *Culiseta annulata* (Schrank) represented 95.4% of engorged females collected on the horse. Only 13 mosquitoes, mainly *Cx. pipiens* and *Cx. modestus*, were collected landing on human bait. *Cx. pipiens* was the only species collected on birds, mainly at the tree-top level.

Host Preferences. *Aedes caspius*, *Ae. vexans*, *An. maculipennis*, and *An. hyrcanus* exhibited a strong mammophilic feeding pattern. However, engorged specimens of all species except *An. maculipennis* also were collected in bird-baited traps in August and at the beginning of September when mosquito densities were peaking (Table 1). Seven (7/9) *Ae. caspius*, 10 (10/12) *Ae. vexans*, and one (1/2) *An. hyrcanus* collected engorged in the bird-baited traps were confirmed to contain avian blood. One *Ae. caspius* had engorged on cattle, one *Ae. vexans* on the horse, and the bloodmeal origin of the other three engorged females was not identified.

At the Tour du Valat site, *Cx. pipiens* was found essentially feeding on birds but also on the horse during September when mosquito densities were high. At the Lunel-Viel site, the feeding behavior of *Cx. pipiens* seemed different; indeed, this species was found to be more opportunistic, with significant numbers found in all traps. In both sites, captures of *Cx. pipiens* on human bait were infrequent. At the Tour du Valat site, *Cx. modestus* fed on birds and also on humans and horses. In contrast, at the Lunel-Viel site, the few individuals of *Cx. modestus* were collected landing on human bait.

Coquillettidia richiardii (Ficalbi) showed an interesting opportunistic feeding behavior with engorged females collected on all hosts (both *Cq. richiardii* collected on birds were confirmed by ELISA test to contain avian blood). Despite the very low densities observed in our survey, this species may be very abundant and even cause nuisance problems in some areas.

Relative Abundance. At the Tour du Valat site, *Ae. caspius* and *Ae. vexans* densities fluctuated with successive peaks of abundance during the capture season (Fig. 3). The peaks were at the end of June, at the end of July, and at the beginning of September, without any apparent relation with rainfall, except perhaps for the last peak of *Ae. caspius* (week 39). This pattern may be explained by artificial flooding of pastures and marshes during summer for agriculture and hunting activities. *An. maculipennis* and *An. hyrcanus* exhibited one peak of abundance at the end of August, after the dry summer (Fig. 3). During the dry summer, rice fields in late maturation may offer large breeding areas for *Anopheles* species. *Cx. pipiens* and *Cx. modestus* were present from July to September, with peak abundance during September (Fig. 3). Other less abundant species in horse collections, *Aedes detritus* (Haliday) sensu lato and *Cs. annulata*, were collected

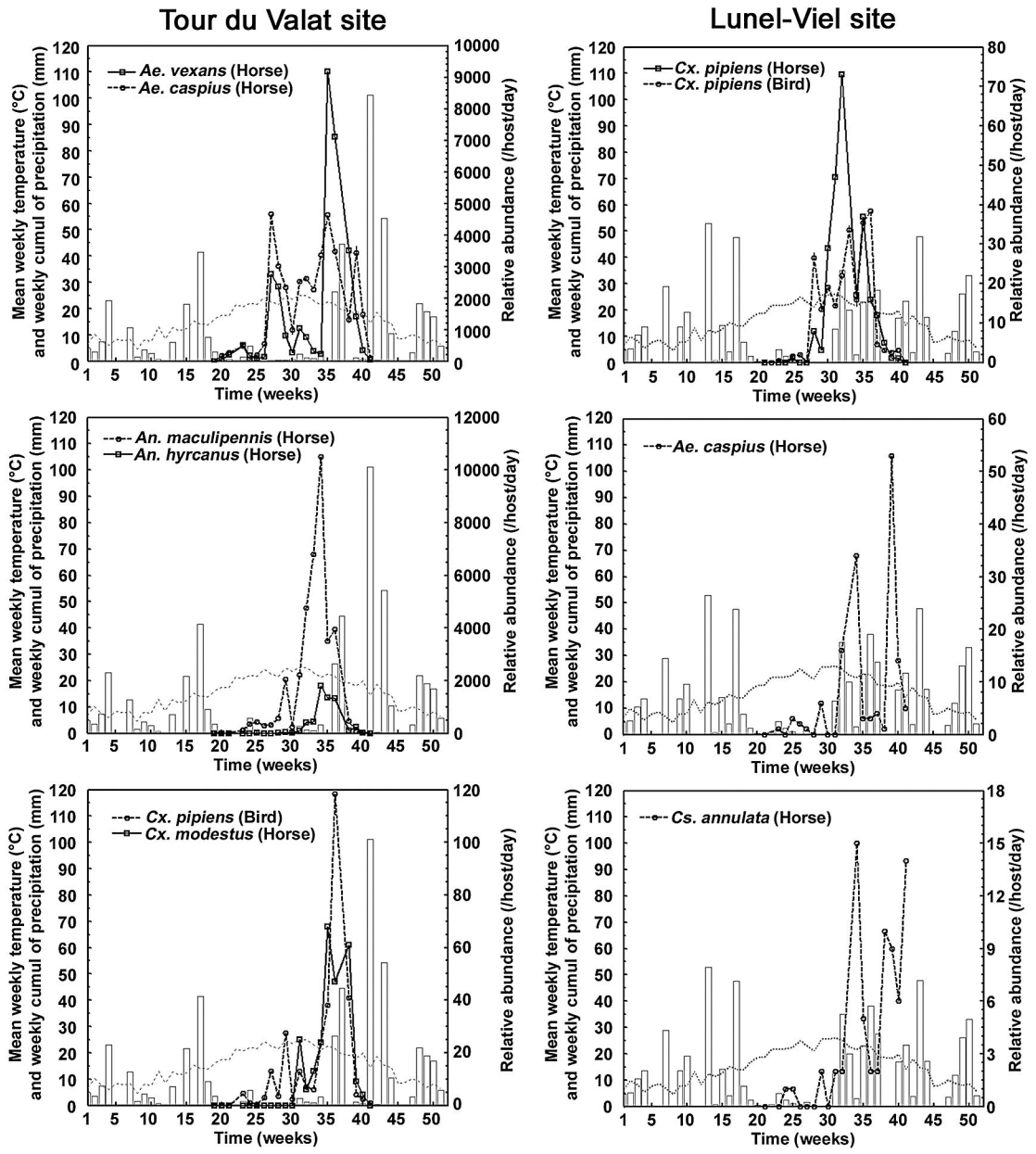


Fig. 3. Weekly relative abundance of dominant species, i.e., the number of engorged females collected during the 24 h of collection on one host (horse or bird: mean of the two traps), at each site (left, Tour du Valat site and right, Lunel-Viel site), plotted with the weekly rainfalls (histogram) and the weekly mean temperatures (dotted gray line).

regularly, with an increased abundance at the end of the capture period.

At the Lunel-Viel site, *Cx. pipiens* was present during July, August, and September, with a peak of abundance in horse collections during August (Fig. 3). *Ae. caspius* exhibited two successive peaks of abundance at the end of August and September (Fig. 3). *Cs. annulata* was captured mainly during the end of the capture period (Fig. 3).

Circadian Biting Activity. Between May and October, the collection interval 4–8 h included the sunrise and the dawn crepuscular period; the collection intervals 8–12, 12–16, and 16–20 h included only diurnal periods (except during October); the collection interval 20–24 h included the sunset and the evening crepuscular period (except during October); and the collection interval 0–4 h included only the nocturnal period (Fig. 4).

Table 1. Diversity and abundance of mosquito species in host-baited collections at the Tour du Valat and the Lunel-Viel sites from May to October, 2004

Host	Biological station of the Tour du Valat			Riding center of Lunel-Viel		
	Species	No. females (no. engorged)	No. males	Species	No. females (no. engorged)	No. males
Horse-baited	*Ae. caspius*	43,517 (39,347)	489	*Cx. pipiens*	583 (251)	39
	Ae. vexans	40,744 (34,897)	1,999	*Ae. caspius*	165 (159)	0
	An. maculipennis s.l.	40,531 (38,000)	32	*Cs. annulata*	78 (72)	0
	An. hyrcanus s.l.	6,663 (5,881)	1	*An. maculipennis* s.l.	16 (15)	0
	Ae. detritus s.l.	566 (493)	2	*An. plumbeus*	8 (7)	0
	Cx. modestus	397 (318)	27	*Cs. longiareolata*	2 (0)	0
	Cs. annulata	361 (326)	0	*Ae. detritus* s.l.	1 (1)	0
	Cx. pipiens	170 (90)	26			
	Cq. richiardii	42 (40)	0			
	Aedes species[a]	26 (26)	0			
	Culiseta subochrea (Edwards)	22 (18)	0			
	An. plumbeus	12 (7)	0			
	Ae. geniculatus	11 (9)	1			
	Aedes annulipes (Meigen)	3 (2)	0			
	Cx. theileri	3 (3)	0			
Bird-baited (≈1 m)	*Cx. pipiens*	695 (419)		*Cx. pipiens*	197 (131)	
	Cx. modestus	134 (104)				
	Ae. caspius	23 (8)	2			
	Ae. vexans	24 (11)				
	An. hyrcanus s.l.	7 (1)				
	Cq. richiardii	2 (2)				
Bird-baited (≈8 m)	*Cx. pipiens*	658 (360)		*Cx. pipiens*	544 (341)	
	Cx. modestus	34 (29)				
	Ae. caspius	11 (1)				
	Ae. vexans	7 (1)				
	An. hyrcanus	1 (1)				
	Cq. richiardii	1 (0)				
	Aedes. species	1 (0)				
Human-baited[c]	*Ae. caspius*	2,055[b]	1	*Cx. modestus*	6	
	Ae. vexans	1,354		*Cx. pipiens*	4	
	An. hyrcanus s.l.	244		*Ae. geniculatus*	2	
	Cx. modestus	75		*Ae. caspius*	1	
	Ae. detritus s.l.	62				
	An. maculipennis s.l.	13				
	An. plumbeus	12				
	Cx. pipiens	5				
	Cq. richiardii	3				
	Ae. geniculatus	1				
	Cs. annulata	1				

[a] Damaged specimens of *Aedes* genus for which identification was not possible.
[b] As soon as possible, females are collected before biting. All females (engorged and unengorged) are considered as aggressive females, thus number of engorged females is not noticed.
[c] As soon as possible, two captors were present for human landing collections.

The biting activity of *Ae. caspius* was clearly diurnal during May, when 88% of engorged females were collected on the horse between 8 and 20 h, with a peak between 12 and 16 h (Fig. 4). After May, engorged *Ae. caspius* were collected on the horse also during the night. However, the minimum number of engorged females was always collected during the interval 0–4 h, representing diurnal activity. The activity of *Ae. vexans* was also diurnal: during all collections, 67% of engorged *Ae. vexans* were collected on the horse between 8 and 20 h. The maximum number of engorged females was always collected between 12 and 16 h and the minimum between 0 and 4 h (Fig. 4).

In contrast, during all collections 85% of engorged *An. maculipennis* and 90% of engorged *An. hyrcanus* were collected on the horse between 20 and 8 h. Engorged *An. maculipennis* were collected during the beginning (20–24 h) and the end (4–8 h) of the night in May, June, September, and October and throughout the night in July and August (Fig. 4), when nighttime temperatures were warmer (>17°C). The biting activity of *An. hyrcanus* started after midnight in July and August and just after sunset (20 h) in September (Fig. 4).

Cx. pipiens exhibited a nocturnal biting activity: during all collections, 96% of engorged females were collected on birds between 20 and 8 h, essentially after midnight (Fig. 4). During the study period, 67% of engorged *Cx. modestus* were collected on birds between 20 and 8 h, with minimum activity at midday (Fig. 4). Surprisingly, 58% of engorged *Cx. modestus* were collected on the horse between 8 and 20 h, with maximum biting activity at midday (35% between 12 and 16 h) and minimum biting activity at midnight (9% between 0 and 4 h).

Discussion

In the current study, 15 of the 24 mosquito species found in Camargue by previous authors (Rioux and Arnold 1955) were collected with horse- and bird-

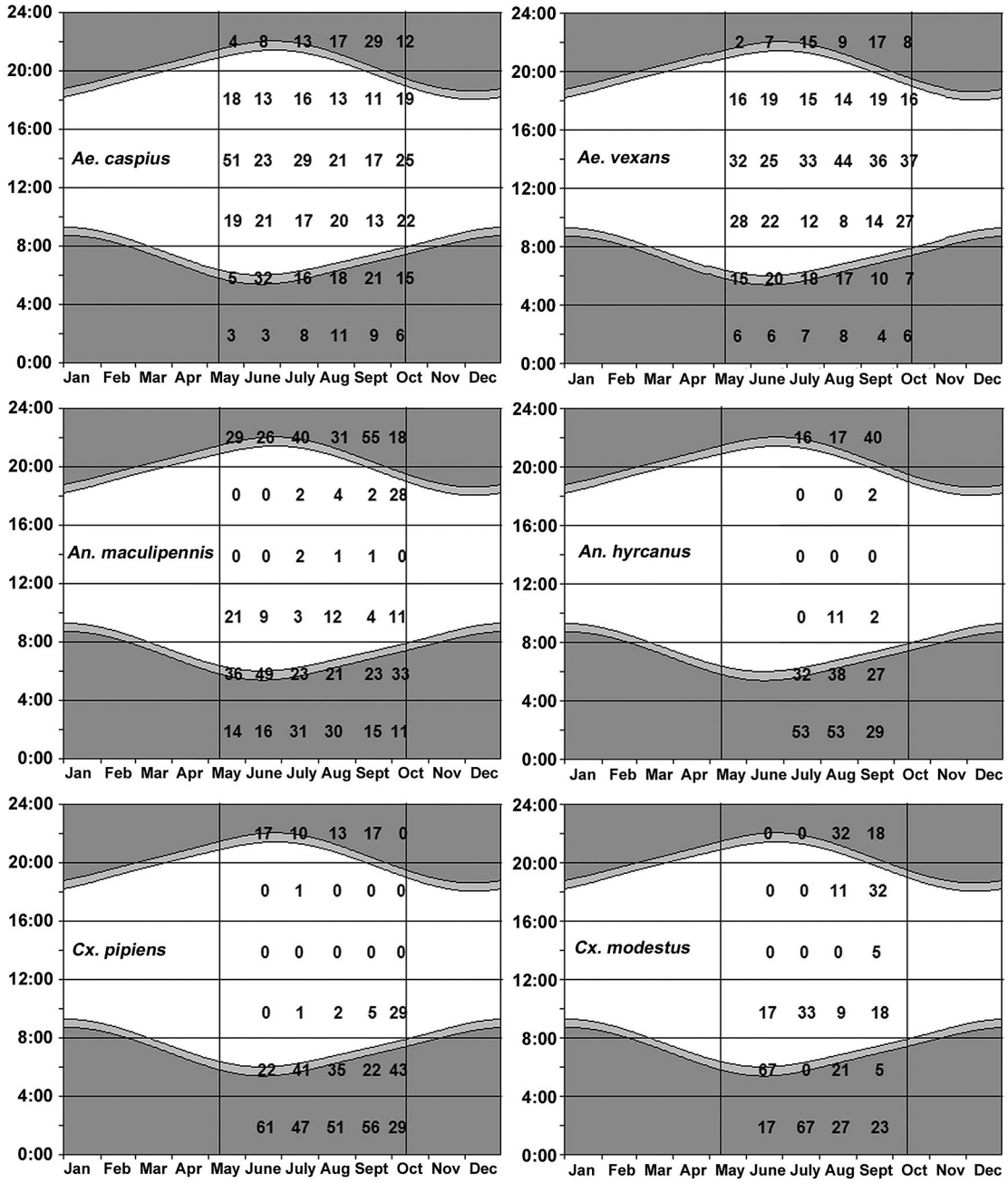


Fig. 4. Monthly percentage of engorged females collected on a given host (in bird-baited traps for *Culex* species; in horse-baited trap for other species) during each collection interval. Time of sunrise and sunset, duration of the day (white), of the night (dark gray), and of the crepuscular period (clear gray) is plotted in background. In each capture month (May to October), the total number of engorged females collected was 265, 1,290, 10,807, 15,395, 8,218, and 1,582 *Ae. caspius*; 191, 901, 6,173, 11,505, 12,022, and 375 *Ae. vexans*; 14, 1,205, 3,175, 27,772, 4,494, and 76 *An. maculipennis*; 0, 3, 38, 4,101, 1,640, and 4 *An. hyrcanus*; 0, 18, 93, 176, 327, and 7 *Cx. pipiens*, and 0, 6, 3, 66, 44, and 0 *Cx. modestus*.

baited traps and human landing collections in the two different ecological sites. The Tour du Valat site produced the greatest diversity with 14 species and the Lunel-Viel site yielded nine species, including two specimens of *Culiseta longiareolata* (Macquart) collected only at this site. At the Tour du Valat site as well as in all wet areas of Camargue, abundant ponds, marshes, flooded meadows, and rice cultures produce

numerous breeding sites. In contrast, in the dry site of Lunel-Viel, breeding sites were almost restricted to artificial breeding containers (as automatic waterers), where larvae of *Cx. pipiens* and *Cs. annulata* were regularly found during our survey. Because of its great dispersal ability, *Ae. caspius* was found in very low densities at the Lunel-Viel site, probably dispersing from littoral breeding sites. Such small densities may result from insecticide spraying against *Ae. caspius* and *Ae. detritus* larvae as carried out routinely in tourist visited littoral areas by the mosquito control agency (Entente Interdépartementale pour la Démoustication du littoral méditerranéen). No spraying activities are carried out in the delta of the Rhône River, including the Tour du Valat site.

The host preferences described in our study are in agreement with the previous descriptions (Rioux 1958), except for the *Culiseta* species that usually are described as bird feeders (Rioux and Arnold 1955, Medlock et al. 2005). Numbers of bites received by a host in a baited-trap may differ from those received in natural conditions, and estimation of the latter is difficult. One expects that an animal-baited trap allowing blood feeding would attract more mosquitoes than a trap that prevents them from feeding on the host (Service 1993). Thus, our results from animal-baited traps allowing blood feeding may overestimate the number of bites received by a host in natural conditions. The finding of engorged mosquitoes with mammal blood in bird-baited traps suggests the need for caution when interpreting collections for animal-baited traps.

In the wet areas of Camargue, the peaks of abundance of the main species were driven mostly by artificial flooding for human activities (cultivation, hunting and fishing) rather than rainfall. Consequently, only the understanding of the complex system of artificial flooding in the delta could explain the dynamics of the different species, especially *Aedes* species. In dry areas of Camargue, the persistence of *Cx. pipiens* throughout the season could be explained by the presence of permanent artificial breeding sites, such as automatic waterers and containers.

Knowledge of the circadian rhythms of mosquito vectors is important to determine the period of contact between hosts and vectors. At the Tour du Valat site, during periods of high mosquito density, collection of all mosquitoes in the horse-baited trap was difficult, in particular during the night (because up to 11,300 individuals were collected in one visit). This difficulty may explain residual activity of some nocturnal species in the morning. Moreover, in temperate regions, the use of constant collection intervals during a long period limits the study's sensitivity to circadian rhythms. Nevertheless, the rhythms observed are in agreement with previous descriptions (Rioux 1958) for the different species. Patterns of mosquito activity are related to the natural daily cycle of light and dark and can fluctuate according to the season (Clements 1999) or daily, for example, under the influence of the temperature or moonlight. *Cx. modestus* was found by Mouchet et al. (1970) to be active throughout the diel, except in the middle of the day in summer and except during the night in fall.

During the 2004 summer, seroconversions of sentinel birds, including at the Tour du Valat site, and an outbreak of 30 confirmed equine cases (including one in the town of Lunel situated very close to Lunel-Viel), showed that WNV circulated close to our study sites (Languille et al. 2005), during the period of mosquito collections.

During past WNV outbreaks in France, seroconversions of sentinel birds and/or equine clinical cases were reported for August, September, and the beginning of October (Joubert et al. 1970, Durand et al. 2002, Del Giudice et al. 2004, Durand et al. 2005, Languille et al. 2005). The period of viral circulation usually spans weeks 30–40, when the most abundant mosquito species collected in our survey are at their peak. *Ae. caspius* and *Ae. vexans* were present in great numbers at the Tour du Valat site during September and could be involved as "bridge" vectors in equine outbreaks in wet area of Camargue. Dispersing from wet areas, *Ae. caspius* may export the virus into dry neighboring zones. *Ae. caspius* infected by intrathoracic inoculation can transmit experimentally WNV (Akhter et al. 1982). Field-collected individuals of *Ae. vexans* have been found positive for WNV in United States (Bernard et al. 2001), where its laboratory vector competence was established (Turell et al. 2000, Sardelis et al. 2001, Goddard et al. 2002). *An. maculipennis* and *An. hyrcanus* peaked at the end of August and then quickly declined during the beginning of September, whereas maximum equine infections were reported during September. *An. hyrcanus* was found engorged on birds, but to our knowledge, this species has never been implicated in WNV transmission. No *An. maculipennis* were collected in bird-baited traps; however, WNV was isolated from field collected individuals of this species in Portugal (Filipe 1972). The number of *Culex* mosquitoes increased progressively during the summer, with a peak in September when the number of WNV clinical cases was greatest. Both *Culex* species have been involved in WNV transmission: *Cx. modestus* in France (Mouchet et al. 1970) and *Cx. pipiens* in Israel (Goldblum et al. 1954), Romania (Savage et al. 1999), and the United States (Bernard et al. 2001). These two species are strongly ornithophilic and good enzootic vector candidates in wet areas of Camargue. However, only *Cx. pipiens* seems able to play this role in the dry zones. *Cx. modestus* aggressively fed on people and horses and is a "bridge" vector candidate in wet areas of Camargue. *Cx. pipiens* (more ornitophilic than *Cx. modestus*) also fed on horses and acts as a "bridge" vector according to some authors (Kilpatrick et al. 2005).

However, the case of *Cx. pipiens* is complicated by the fact that the *Cx. pipiens* taxon is divided in two forms in Europe: the *pipiens* form, described as anautogenous, eurygamous and mainly ornithophilic and the *molestus* form, described as autogenous, stenogamous, and strongly anthropophilic (Vinogradova 2000). Behavioral and physiological differences between the two forms are considered as rapid ecolog-

ical adaptations of the form *pipiens* to rural open air (anautogenous) and of the form *molestus* to urban hypogenous (autogenous) breeding sites (Rioux 1958, Barr 1981, Harbach et al. 1984). However, the existence of two species is still debated (Fonseca et al. 2004). At both capture sites, *Cx. pipiens* individuals develop in open air habitats and belong to the *pipiens* form. Stenogamous and completely anautogenous populations breeding in open air sites were described in southern France with anthropophilic (Rioux et al. 1965) or ornithophilic (Pasteur et al. 1977) preferences. Physicochemical characteristics (especially degree of organic pollution) of breeding sites were found associated with the degree of mammophily of *Cx. pipiens* populations in southern France (Gabinaud et al. 1985). In both sites, only nine *Cx. pipiens* were collected landing on human bait. A better knowledge of the distribution, dynamics, and host preferences of *Cx. pipiens* is an important point to better understand its potential role in WNV transmission in France.

Among other lower densities species collected in our survey, vector competence was established for *Cs. longiareolata* in Egypt (Hurlbut 1956), *Aedes geniculatus* (Olivier) and *Anopheles plumbeus* Stephens in France (Vermeil et al. 1960), and *Culex theileri* Theobald in South Africa (Jupp et al. 1966). WNV was found in field collected individuals of *Cx. theileri* (McIntosh et al. 1967) and *Cq. richiardii* (Bashkirtsev et al. 1969).

These host-baited collections established the abundance and tendencies of bird-, horse-, and human-feeding species in two sites of Camargue during a WNV epizootic. The next requirement to confirm these mosquito species as WNV vectors is to conduct vector competence tests.

Acknowledgments

We thank O. Pineau, M. Gauthier-Clerc, and the staff of the Station Biologique de la Tour du Valat for logistic help and scientific collaboration; B. Cotton and M. Deydier for loaning the horse and ducks; and M. Leduc, M. Chanteau, and C. Rotela for help in mosquito collections. We thank F. Schaffner, C. Lagneau, and the staff of the Entente Interdépartementale pour la Démoustication du littoral méditerranéen for logistic and technical support and scientific collaboration; D. Fontenille, N. Ponçon, and the staff of the Laboratoire de lutte contre les Insectes Nuisibles (Institut de Recherche pour le Développement) for bloodmeal analyses; and H. Zeller of the Centre National de Référence des Arboviroses et Fièvres Hémorragiques (Institut Pasteur) for scientific collaboration. We thank P. M. Bouquet, Ecole Nationale Supérieure d'Agronomie (ENSA-Montpellier) in Salon de Provence, and staff for logistic help and M. Boccoz and M. Jouan of the Institut Pasteur for supporting this research project. The ethical committee of the Ecole Nationale Vétérinaire de Lyon gave approval for this protocol (decision 04/14) and for all mosquito collection methodologies included in this study. Informed consent of collectors participating to the human landing collections was obtained.

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Received 30 September 2005; accepted 28 March 2006.