Experimental vectorial capacity of arbovirus-infected mosquitoes

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Topics

1. Dengue virus in mosquitoes
2. West Nile virus in mosquitoes
3. Effective mosquito countermeasure

1.Dengue virus in mosquitoes

A. Is only *Aedes albopictus recognized* as a dengue vector in Japan?

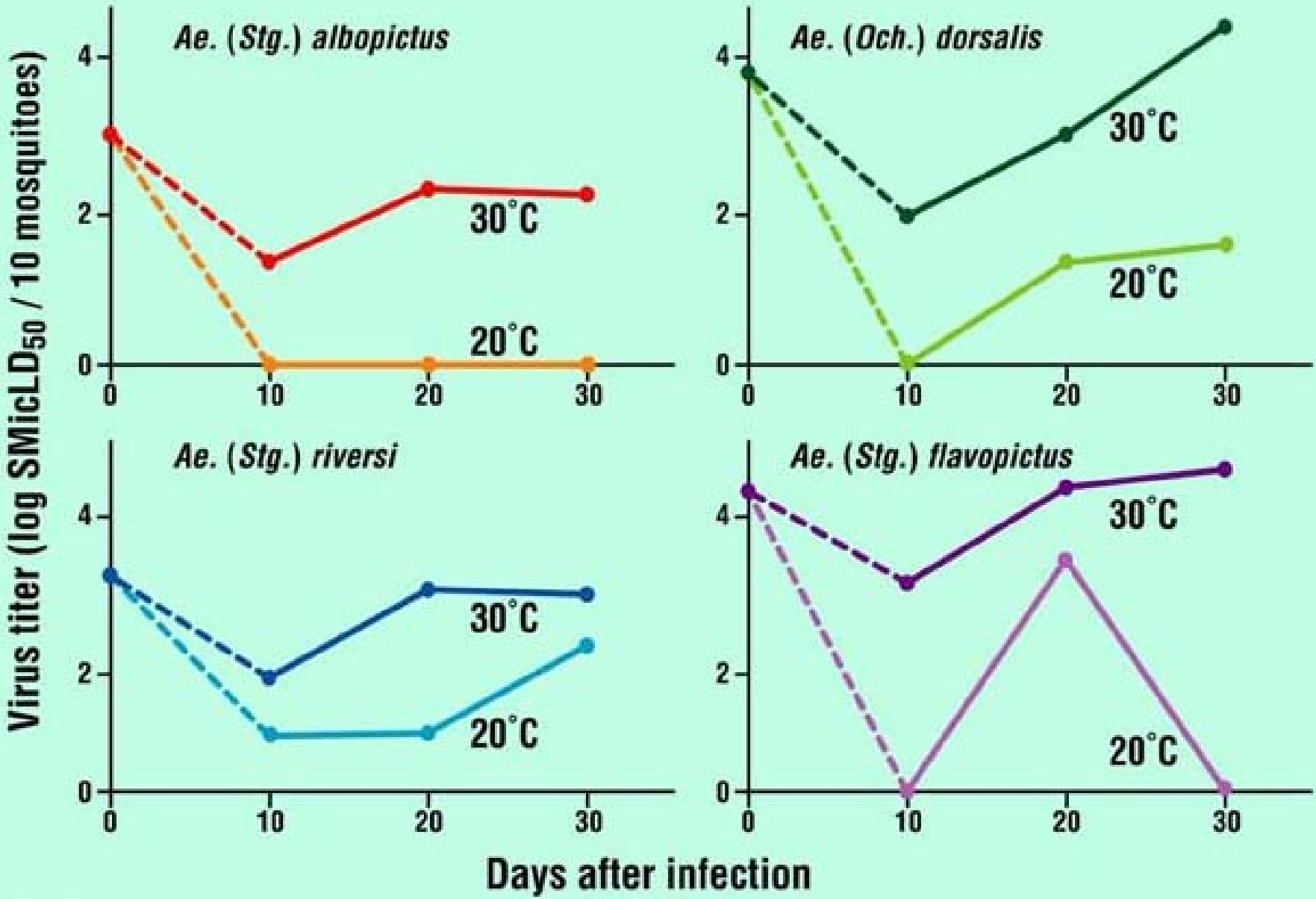
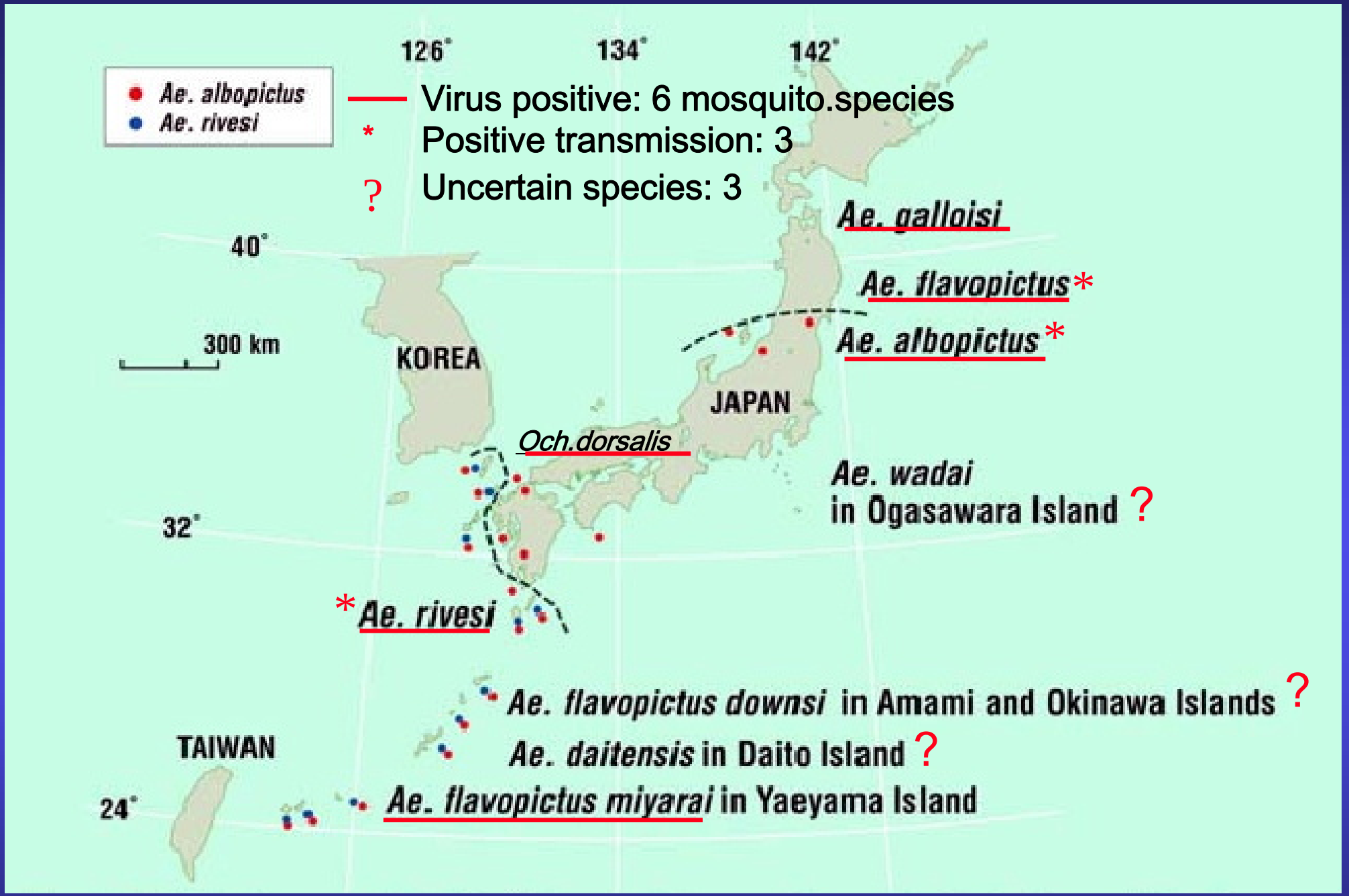


Fig. 3 Comparative growth curve for dengue-2 virus in 10-pooled Japanese mosquitoes at intervals after oral infection at 20 and 30˚C, respectively



Distribution of 9 putative dengue vectors in Japan

1. Dengue virus in mosquitoes

C. How dengue viruses are maintained in tropical endemic areas?

Fig. Mosquito collection at a dengue patient's house in Thailand

Table 1 Dengue infection status of mosquitoes collected inside houses at #1 village, Sansuk district, Chonburi Province, Thailand

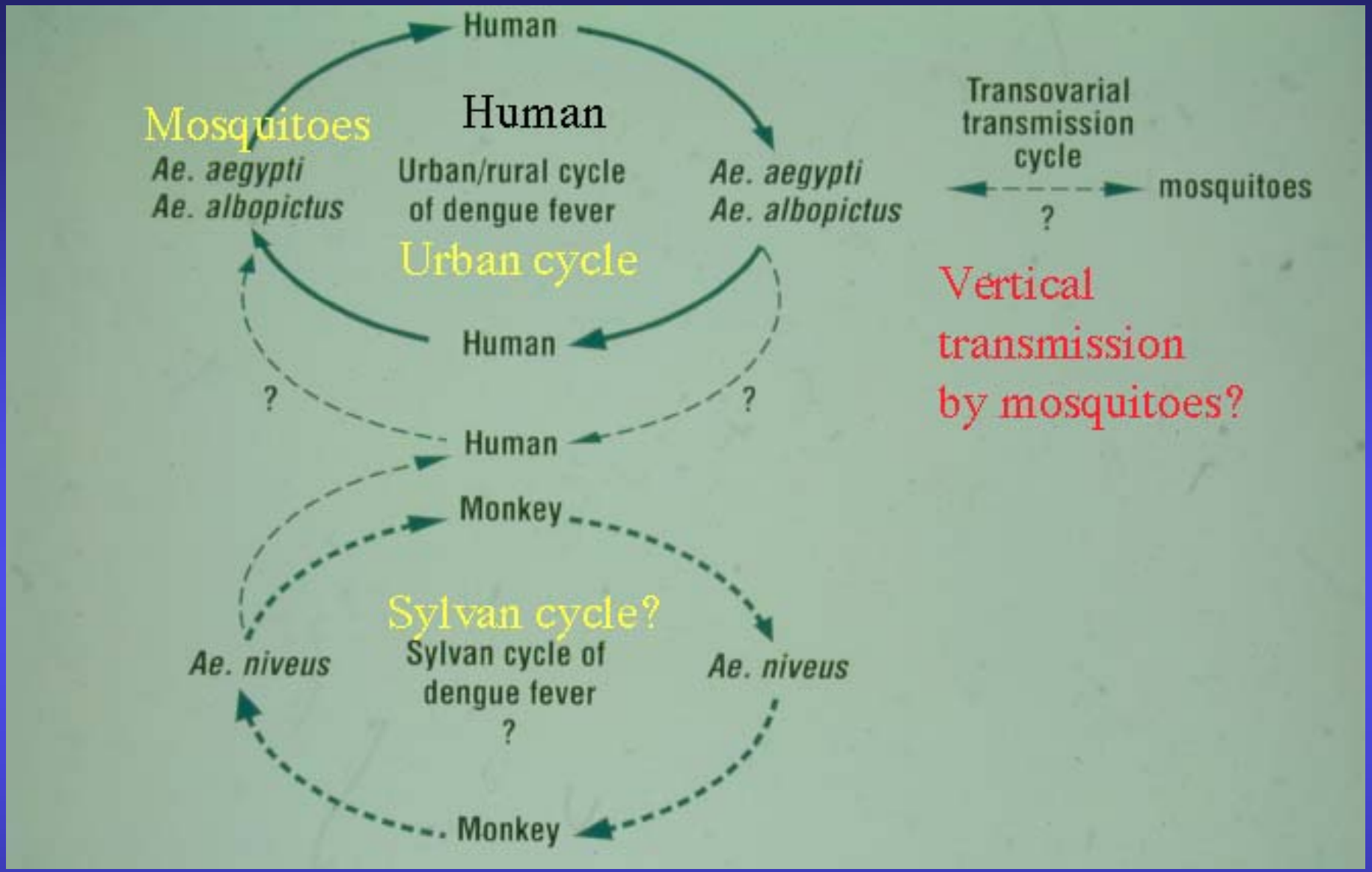
Patient's house number	Mosquito species collected inside houses		
	Aedes aegypti	*Ae.albopictus*	Other mosquitoes
2003.0721.1	0♀+1♂*/1♀+1♂ (0%,100%)**	0/0(0)	0/0(0)
2003.0721.2A	0♀+0♂ /5♀+1♂ (0%, 0%)	0/0(0)	0/0(0)
2003.0721.2B	0♀+0♂ /4♀+1♂ (0%, 0%)	0/0(0)	?♂/1♂ (?%) Cx.q.***
2003.0721.2C	1♀+1♂ /9♀+25♂(11%, 4%)	0/0(0)	?♀+?♂/2♀+1♂(?%,?%)Cx.q
2003.0721.3A	0♀+?♂ /1♀+2♂ (0%, ?%)	0/0(0)	?♀/1♀(?%)Cx.q,?♀/1♀(?%)Mn.
2003.0721.3B	1♀+?♂ /4♀+4♂ (25%, ?%)	0/0(0)	?♀/15♀(?%)Cx.q.
2003.0721.3C	?♂ / 1♂ (?%)	0/0(0)	?♀+?♂/1♀+1♂ (?%,?%) Cx.q.
2003.0721.3D	1♀+?♂ /4♀+2♂ (25%, 0%)	0/0(0)	?♂/4♂ (?%) Cx.q.
2003.0721.3E	0♀+?♂ /1♀+12♂(0%, ?%) ?♀/7♀(?%)Mn.	0/0(0)	?♀+?♂/7♀+18♂(?%,?%)Cx.q.,
2003.0721.4A	?♂ / 1♂ (?%)	0/0(0)	0/0(0)
2003.0721.4B	1♀ /1♀ (100%	0/0(0)	0/0(0)
2003.0721.5A	0/0	0/0(0)	0/0(0)
2003.0721.5B	?♂ / 1♂ (?%)	0/0(0)	?♀/2♀ (?%) Cx.q.
2003.0721.6A	0/0(0)	0/0(0)	?♀+?♂/7♀+4♂ (?%,?%) Cx.q.
Total	4♀+2♂*/30♀+51♂(13%,3.9%)**		

All tested mosquitoes were maintained at 25C for 14 days, and lived adults only used for RT-PCR.

* Positive number of mosquitoes with dengue viral genome / Number of mosquitoes collected

** Percentage (%) of mosquitoes with dengue viral genome by RT-PCR

*** Cx. q. = *Culex quinquefasciatus*, Mn = *Mansonia* species



Transmission cycle of dengue viruses maintained by arthropod and vertebrates in nature
(broken lines show indefinite cycle)

2.West Nile virus in mosquitoes

A. Are Japanese mosquitoes able to transmit West Nile virus?

No. of collected mosquitoes: 17,243

Cx.p.pallens : 14,071(81,6%)

Ae.albopictus: 2,287(13,3%)

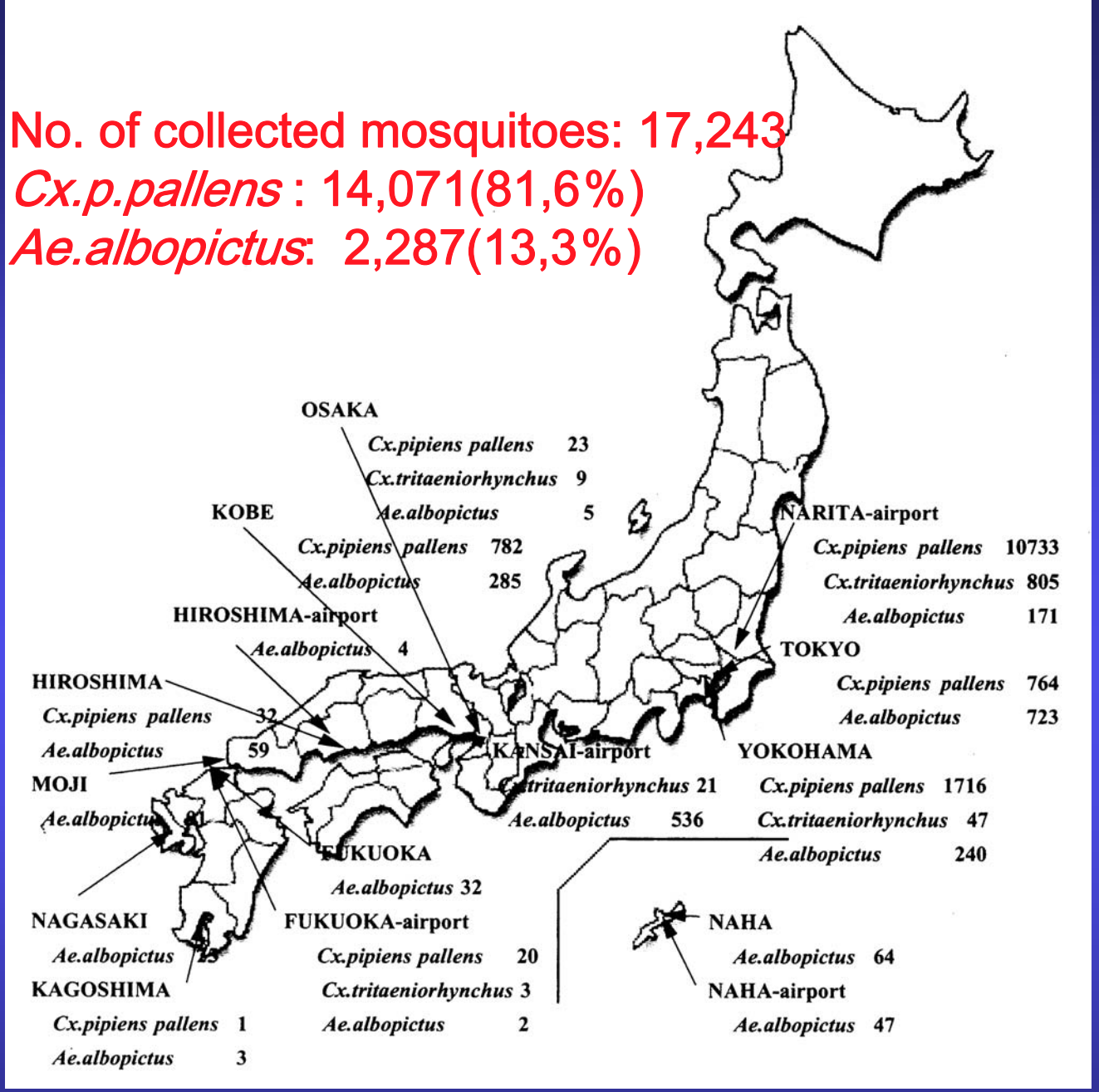
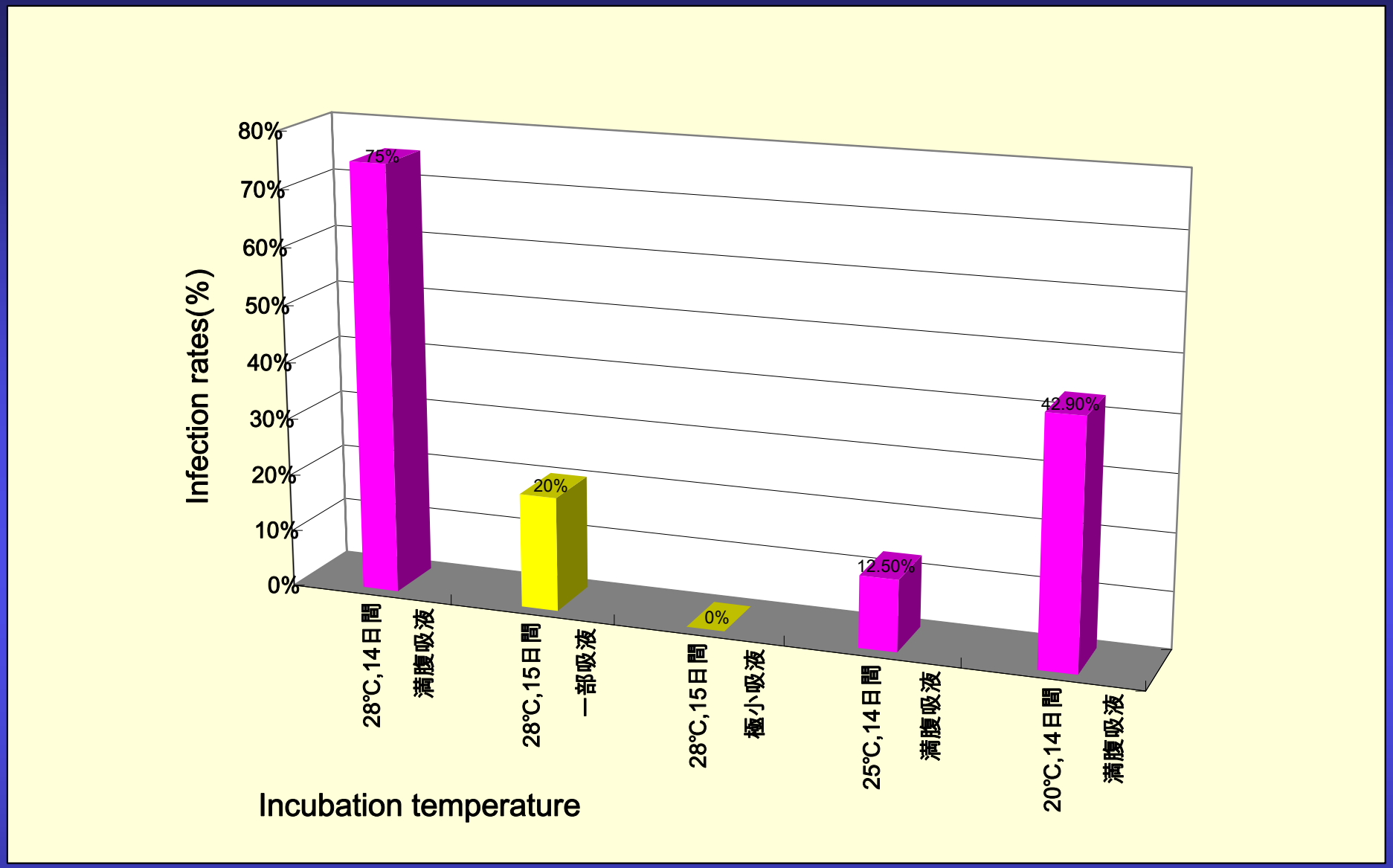


Fig.1 Comparative number and species of mosquitoes collected by light trap (Data from Quarantine Station, 2001)



Virus infection rates of orally-infected *Cx. p. pallens* under the different temperature conditions.

[28℃:感染蚊6/供試個体8(75%),25℃:1/8(12.5%),20℃:3/7(42.9%)]

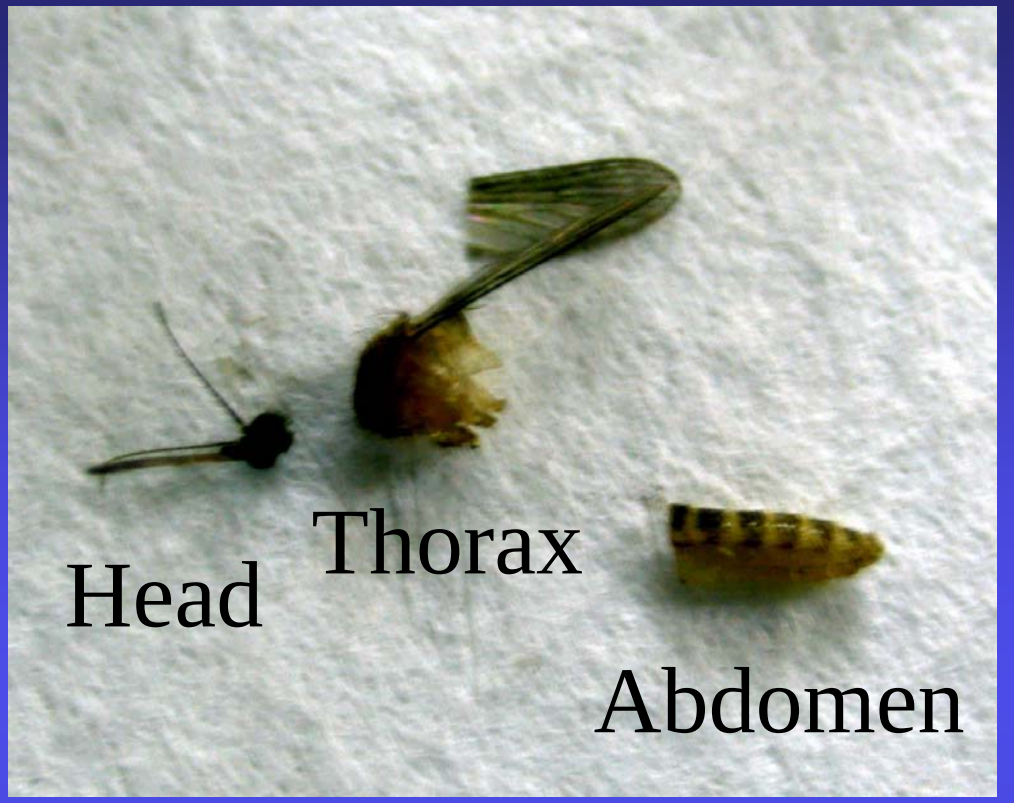
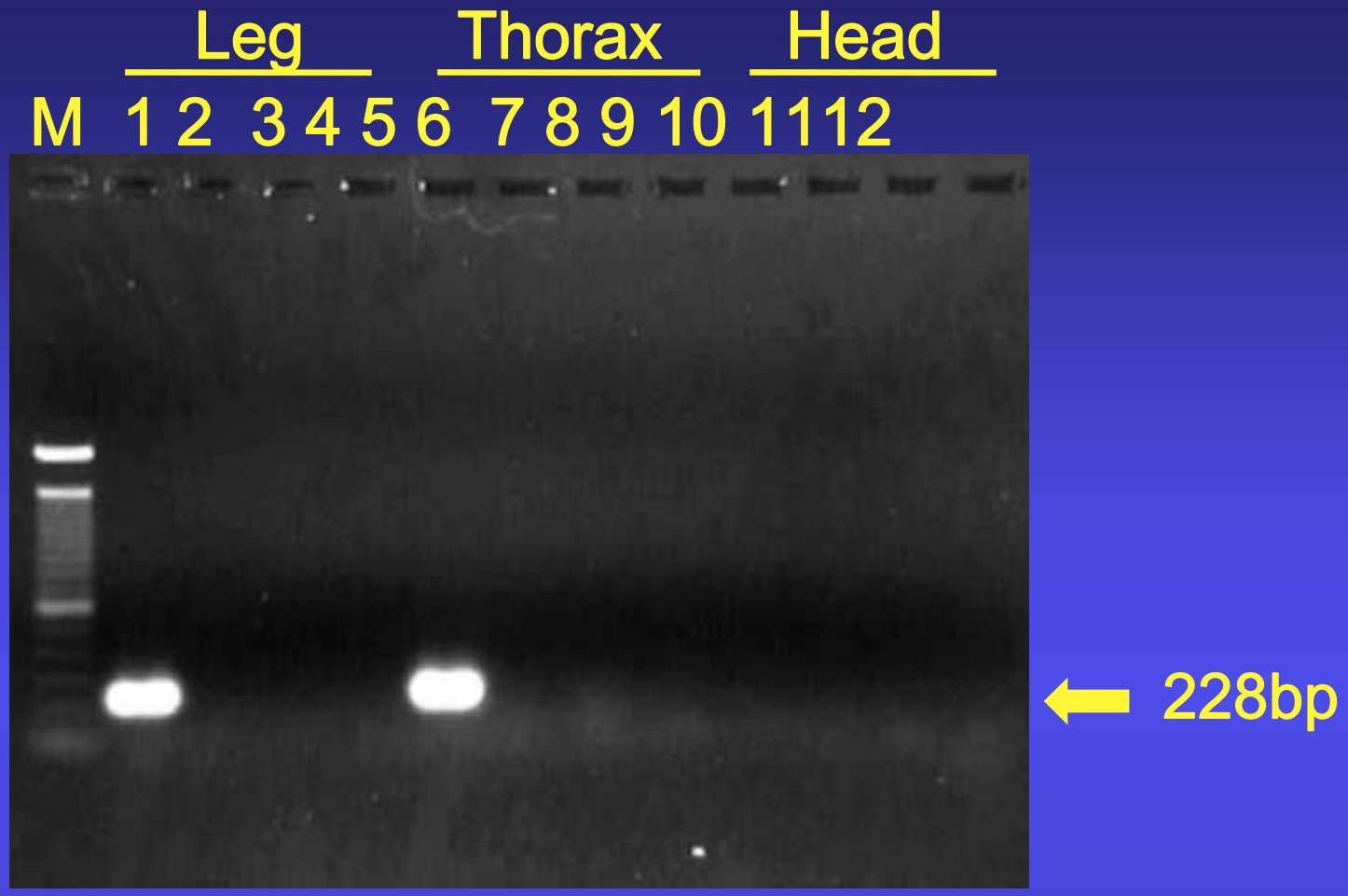


Fig. Female mosquito of *Culex pipiens pallens*

Each part of the mosquito was separated in order to examine the existence of the West Nile viral genome by the RT-PCR reaction.



RT-PCR amplification of West Nile viral genome in partial mosquito leg, thorax and head, respectively, derived from four mosquito abdomens with viral genome-positive.

(Each template RNA was extracted by Qiagen system)

Table 2 Detection of West Nile virus genome in blood of mice bitten/engorged by intrathorathically *infected Culex pipiens pallens* female mosquitoes post 14 days

	030604.1	030604.1	030604.2	030604.2	030604.3	030604.4	030604.8	030604.7
Days post infection	1	2	3	4	5	6	7	8
1	no sample	no sample	no sample	no sample	no sample	no sample	no sample	no sample
3	±*(no symptom)	,-(sick?)	NT	NT	NT	NT	NT	NT
5	,-	,-(sick)	, -	NT	NT	NT	NT	NT
7	, -	?(dead)	±(sick,blood)*	NT	NT	NT	NT	NT
8	, -			NT	NT	NT	NT	NT
10	-(sick,blood)			NT	NT	NT	NT	NT
17				NT	NT	NT	NT	NT

±: Very faint WNV specific band was observed by RT-PCR, - : No band of RT-PCR product, NT: Not tested

*:Homologous sequences of RT-PCR product to that of WNV Nigeria strain (NCBI accession no.,NM12294.2)

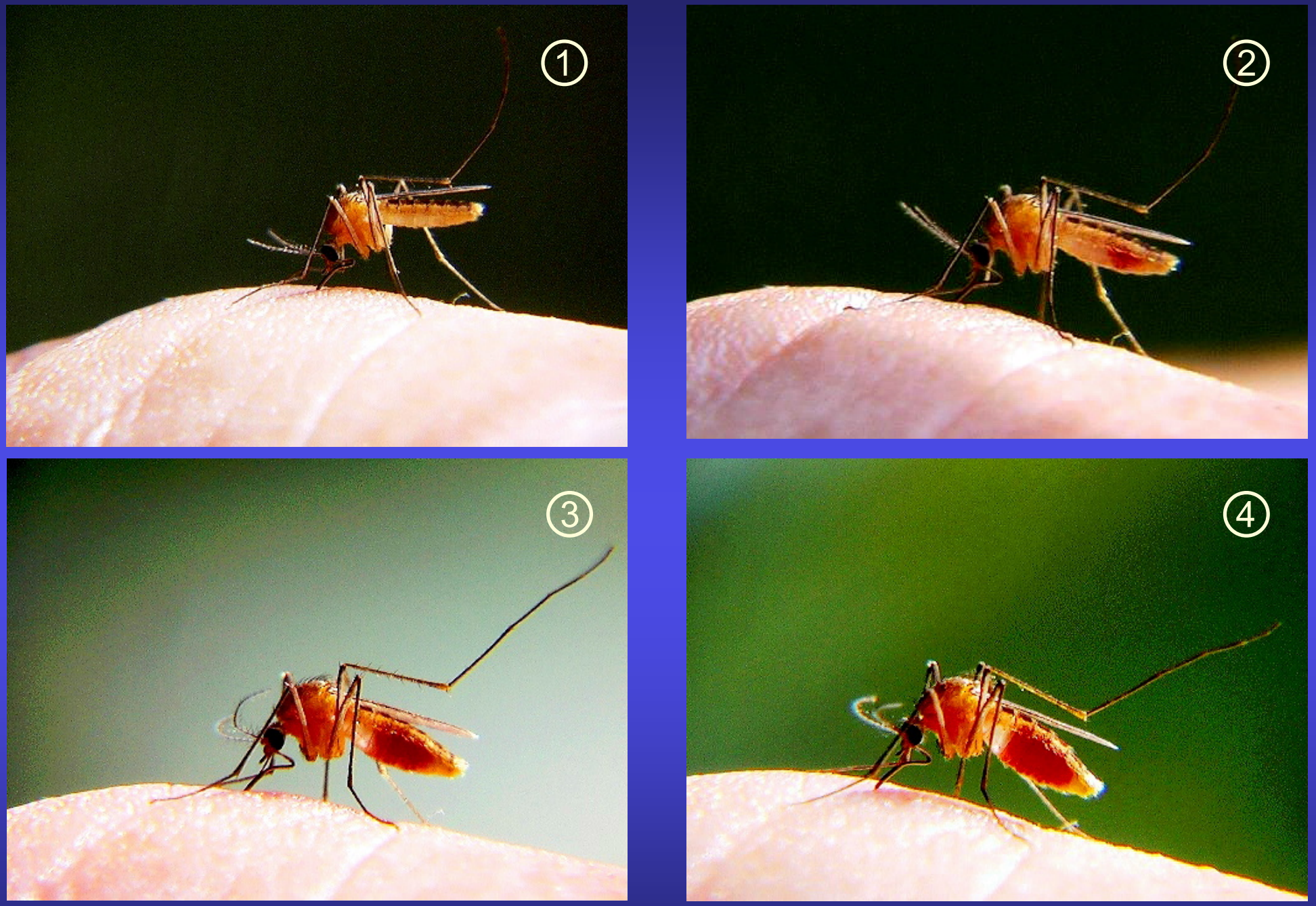


Fig. 1 *Culex inatomii* female engorged blood on a human finger

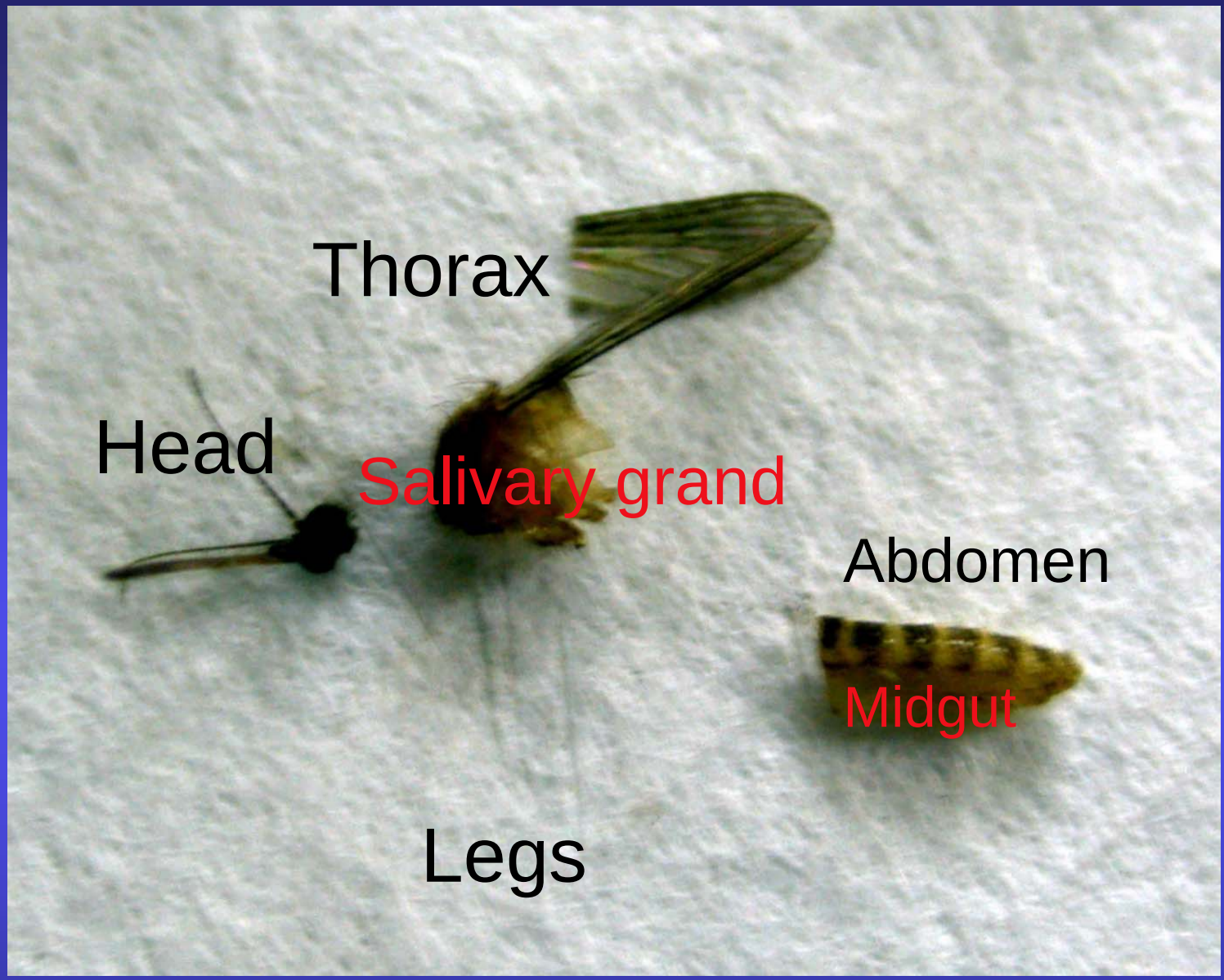


Fig.3 Head, thorax with salivary grand, abdomen with midgut, and legs of *Culex inatomii* female mosquito

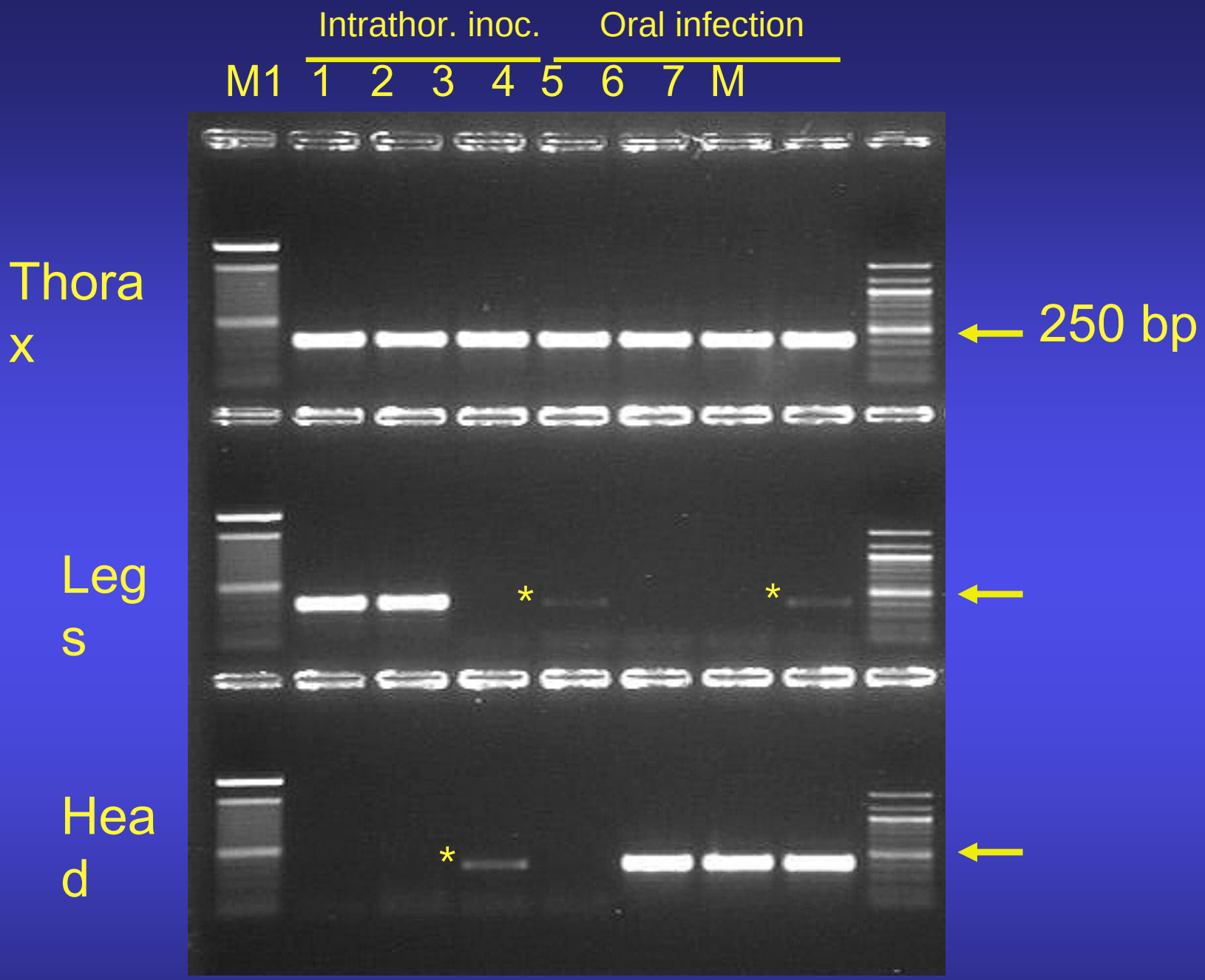


Fig. 4b Detection of WNV genome from thoraxes, legs and heads of *Culex inatomii* by intrathoracic and oral infection, respectively. (M: 100bp ladder marker, Primer2: WNNY514V-E, WNNY904-E)

Table Symptom, RT-PCR with total RNA derived from internal organs, and neutralization antibody in blood of mice bitten by West Nile virus-infected mosquitoes

Mosquito number	Symptom of mouse	RT-PCR	NT*	
			WN**	JE***
1.1	+ + +	+	640	40
1.2	+ + + +	-	640	40
2.1	±	+	160	40
2.2	-	-	40	20
3.1	+	+	640	40
3.2	-	+	640	40
4.1	+ +	+ +	640	40
4.2	-	faint band	40	40

* Titer of neutralization antibody
** NT antibody to West Nile virus
*** NT antibody to Japanese encephalitis virus

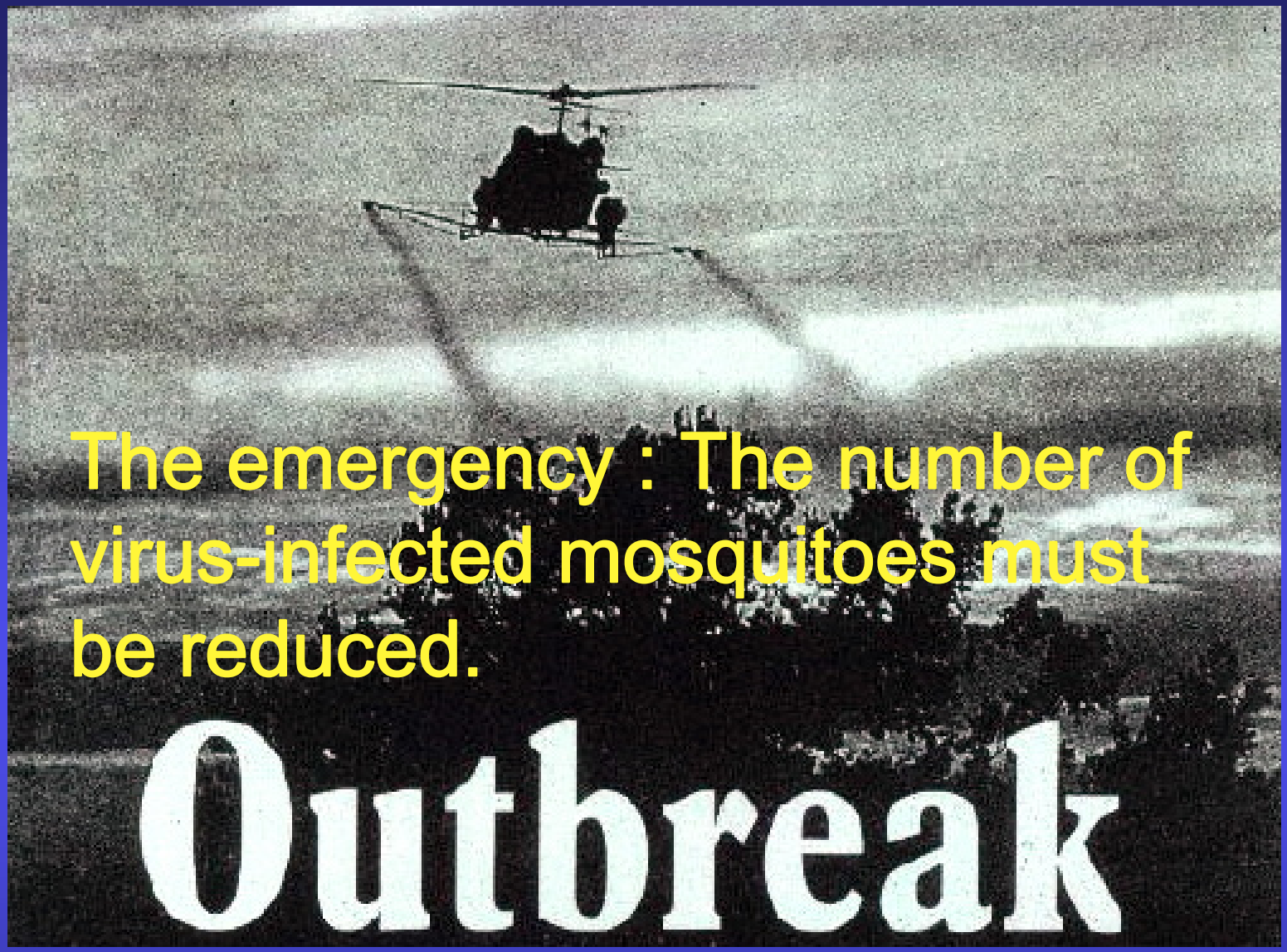
Putative Japanese vector mosquitoes related to West Nile virus

Japanese mosquito species	Experimental infection	Experimental transmission
Culex pipiens pallens	yes	yes
Culex p. molestus	yes	NT
Culex quinquefasciatus	NT	NT
Culex inatomii	yes	yes
Aedes albopictus	yes	yes
Och. japonicus	yes	NT
Ae. vexans	NT	NT
Och. togoi	NT	NT

NT: not tested

3. Effective mosquito countermeasures

a. How to fight and cope against the vector-born diseases in non-endemic area?



Adult mosquito countermeasure in vector-born disease epidemic by insecticide spraying. (Organized by Country, administrative divisions).

Individual defense

Source reduction of mosquito larvae in artificial small containers at the neighborhood association level.

Sorry!
Sold out!
Mosquito coil

Aedes mosquitoes may come with accompanying the dengue virus every daytime.


Ae.albopictus

Culex mosquitoes may come with accompanying the West Nile virus every night.

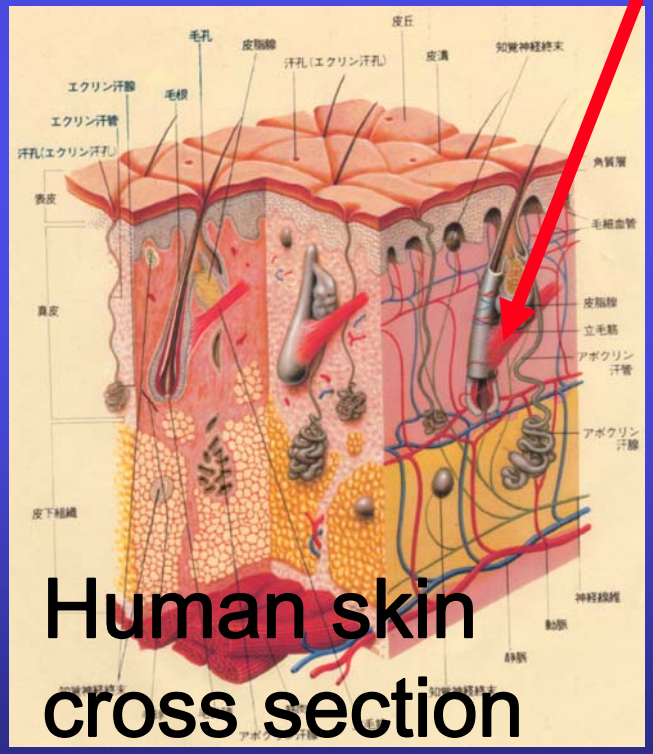

Culex mosquitoes

Threat of infectious diseases comes in forgotten time

Collaborators on dengue and West Nile virus research

S.Raweewan,N.Komalamisra,S.Leemingsawat,
Y.Rongsriyam (Mahidol Univ.,Thailand)
T.Takassaki,I.Kurane (NIID, Tokyo)
I.Takashima (Hokkaido Univ.)
S.Imura,N.Uchida (Kobe Quarantine)
H.Ushijima (Tokyo Uinv.)

Dengue or West Nile virus inoculation by blood-feeding mosquito



Lymph duct infection

↓

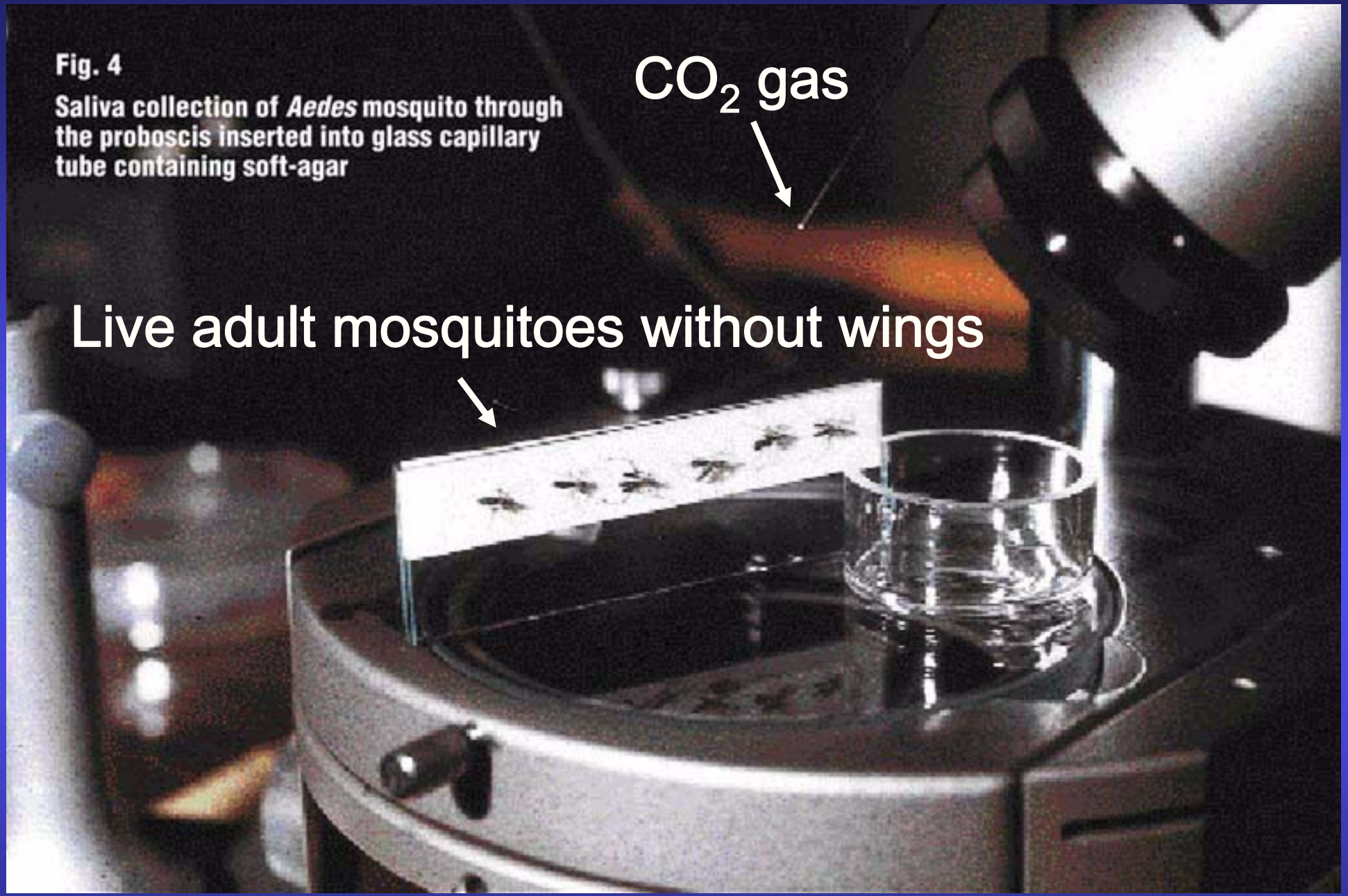
Involvement of dendritic cells?

↓

lymph node infection

↓ High fever

Blood infection

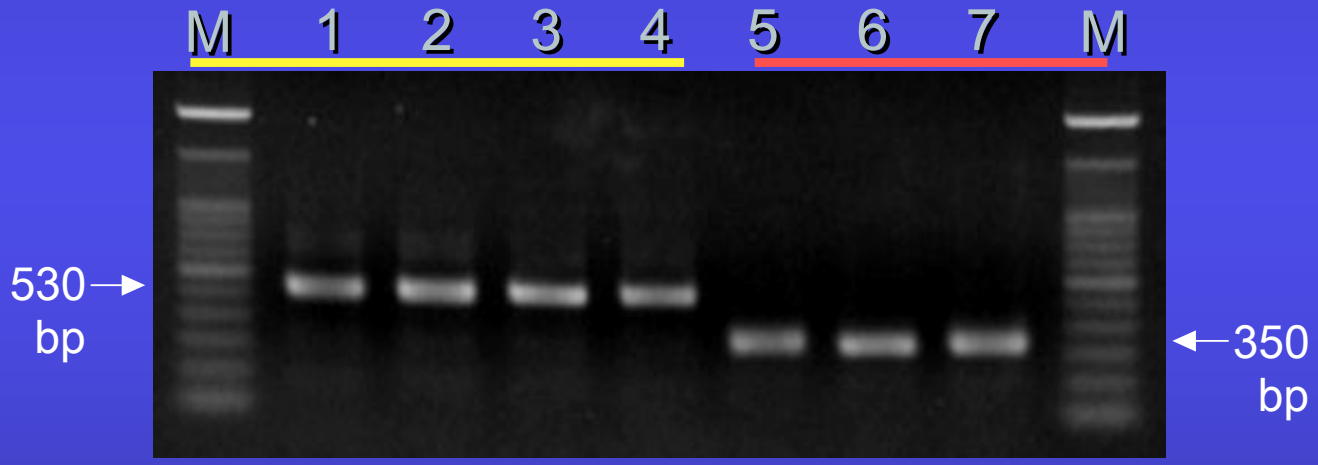


Collection of mosquito saliva for dengue virus

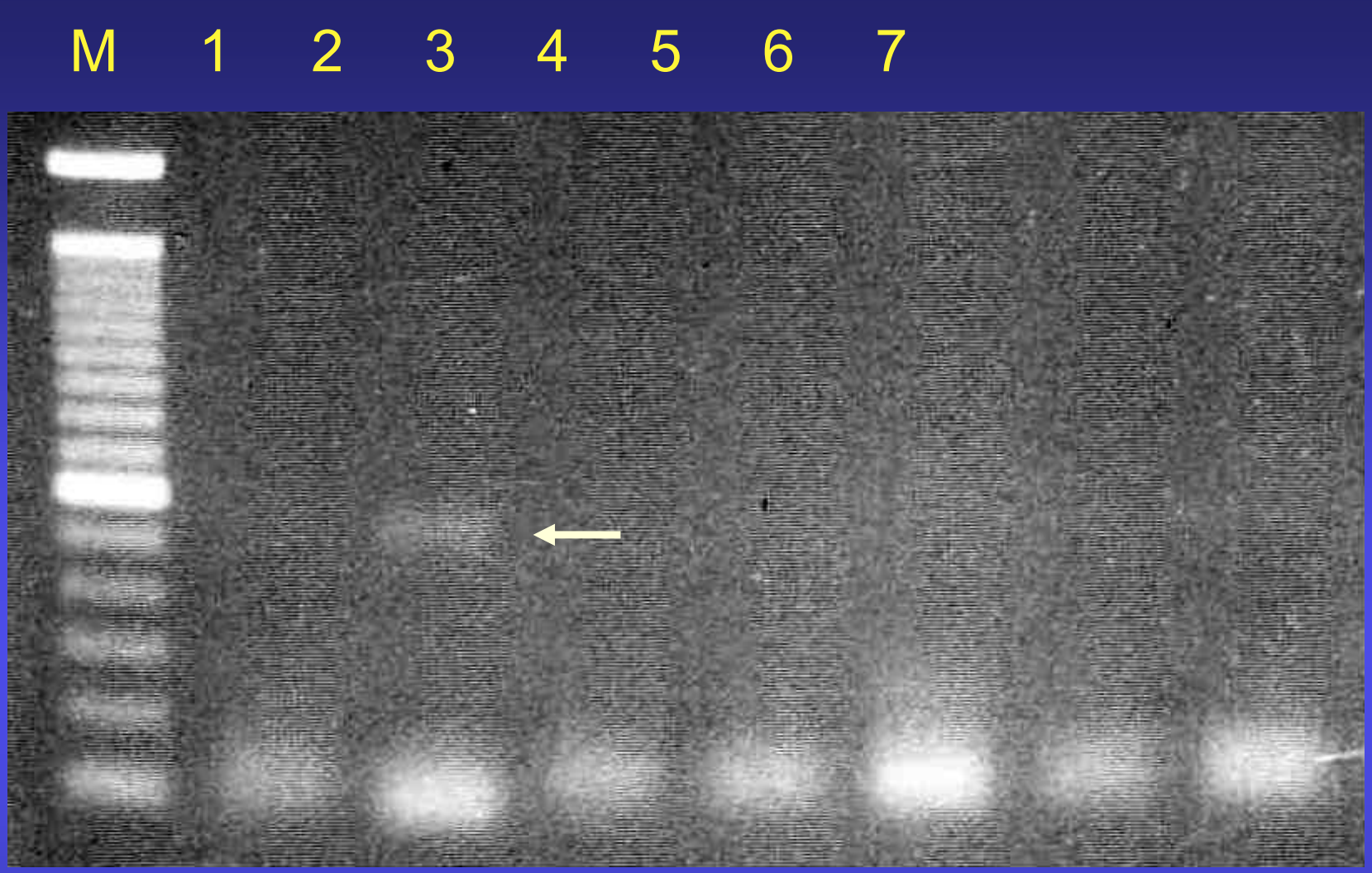
Virus inoculation into adult mosquitoes in a closed box at BSL 3

1. Dengue virus in mosquitoes

B. Is Japan free from the dengue vector, *Aedes aegypti*?



Different number of nucleotides in internal transcribed spacer 2 (ITS 2) in rDNA cistron between the both mosquito species



Amplification of dengue viral genome from individual *Aedes aegypit* mosquitoes collected in the patient house. (Arrow shows PCR positive male.)

Table List of mosquito species with West Nile virus

1	*Aedes albopictus*	21	*Cx. erraticus*
2	*Ae. aegypti*	20	*Cx. territans*
3	*Ae. cinereus*	23	*Culiseta melanura*
4	*Ae. vexans*	24	*Cs. inornata*
5	*Anopheles atropos*	25	*Deinocerites cancer*
6	*An. barberi*	26	*Ochlerotatus atlanticus*
7	*An. bradleyi*	27	*Och. atropalpus*
8	*An. crucians*	28	*Och. canadensis*
9	*An. puncitpennis*	29	*Och. cantator*
10	*An. quadrimaculatus*	30	*Och. japonicus*
11	*An. walkeri*	31	*Och. sollicitans*
12	*Coquillettidia perturbans*	32	*Och. taeniorhynchus*
13	*Culex pipiens molestus*	33	*Och. tormentor*
14	*Cx. pipiens pallens*	34	*Och. triseriatus*
15	*Cx. pipiens pipiens*	35	*Och. trivittatus*
16	*Cx. pipiens quinquefasciatus*	36	*Orthopodomyia signifera*
17	*Cx. restuans*	37	*Psorophora columbiae*
18	*Cx. salinarius*	38	*Ps. ciliata*
19	*Cx. tarsalis*	39	*Ps. ferox*
20	*Cx .nigripalpus*	40	*Uranotaenia sapphirina*

(modified from CDC data, USA)

Culex pipiens pallens (Female and male)